

# TOXICITY TESTING RESULTS

**ALON ASPHALT COMPANY  
SEATTLE, WASHINGTON**

**AUGUST 2023 WET**

**Prepared for**

Alon Asphalt Company  
Richmond Beach Terminal  
20555 Richmond Beach Drive NW  
Seattle, WA 98177

**Prepared by**

EcoAnalysts, Inc.  
Port Gamble Laboratory  
PO Box 216  
4770 NE View Drive  
Port Gamble, WA 98364

**NPDES Permit No.:** WA0003239

**Report ID:** PG1801.01

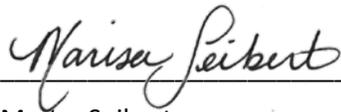
**Submittal Date:** September 29, 2023



Accredited in accordance with  
NELAP, ORELAP ID 4165

All testing reported herein was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and EcoAnalysts is not responsible for use of less than the complete report. The test results summarized in this report apply only to the sample(s) evaluated. This document is uncontrolled when printed or accessed from electronic distribution.

Approved By

A handwritten signature in cursive script that reads "Marisa Seibert". The signature is written in black ink and is positioned above a horizontal line.

---

Marisa Seibert

Program Manager

Author:

Julia Levensgood

QA Review:

Mary Ann Rempel-Hester

## CONTENTS

<b>1.</b>	<b>EXECUTIVE SUMMARY</b>	<b>1</b>
<b>2.</b>	<b>METHODS</b>	<b>1</b>
2.1	Sample Collection and Storage	1
2.2	Bioassay Testing	2
2.3	Organisms for Testing	2
2.4	Water for Bioassay Testing	3
2.5	Sample Adjustment	3
2.6	Data Management and Analysis	3
2.7	Quality Assurance/Quality Control	3
<b>3.</b>	<b>RESULTS</b>	<b>4</b>
3.1	Mysid ( <i>Americamysis bahia</i> ) Acute Test Results	4
3.2	<i>Dendraster excentricus</i> Test Results	6
3.3	Topsmelt ( <i>Atherinops affinis</i> ) Acute Test Results	8
3.4	Topsmelt ( <i>Atherinops affinis</i> ) Chronic Test Results	10
<b>4.</b>	<b>REFERENCES</b>	<b>13</b>

## TABLES

Table 1-1. Toxicity Test Results Summary .....	1
Table 1-2. Permit Compliance Results .....	1
Table 2-1. Sample Conditions upon Receipt.....	2
Table 2-2. Biological Testing Performed.....	2
Table 2-3. Salinity Adjustment of Project Samples.....	3
Table 3-1. Endpoint Summary for <i>Americamysis bahia</i> Acute Test.....	4
Table 3-2. Test Condition Summary for <i>Americamysis bahia</i> Acute Test.....	5
Table 3-3 Results Summary for <i>Dendraster excentricus</i> Survival and Development Test.....	6
Table 3-4. Test Condition Summary for <i>Dendraster excentricus</i> Survival and Development Test.	7
Table 3-5. Endpoint Summary for <i>Atherinops affinis</i> Acute Test .....	8
Table 3-6. Test Condition Summary for <i>Atherinops affinis</i> Acute Test .....	9
Table 3-7. Endpoint Summary for the <i>Atherinops affinis</i> Chronic Test .....	10
Table 3-8. Statistical Results Summary for <i>Atherinops affinis</i> Chronic Test.....	11
Table 3-9. Test Condition Summary for <i>Atherinops affinis</i> Chronic Test .....	12

## APPENDICES

- Appendix A: Statistical Comparisons and Laboratory Documents  
Appendix B: Chain of Custody, Sample Receipt Forms

## ACRONYMS AND ABBREVIATIONS

ABS	Aquatic BioSystems, Inc.
ACEC	Acute Critical Effluent Concentration
CCEC	Chronic Critical Effluent Concentration
EPA	Environmental Protection Agency
LC <sub>50</sub> /EC <sub>50</sub>	Lethal/Effect Concentration to 50% of Test Population
LOEL	Lowest Observed Effect Level
mg/L	Milligrams per Liter
NOEL	No Observed Effect Level
NPDES	National Pollutant Discharge Elimination System
QM	Quality Manual
SOP	Standard Operating Practices
WDOE	Washington Department of Ecology
WET:	Whole Effluent Toxicity

## 1. EXECUTIVE SUMMARY

EcoAnalysts conducted Whole Effluent Toxicity (WET) testing on three effluent samples collected by Alon Asphalt Company personnel as part of the effluent characterization. The objective of this program was to assess the potential toxicity of primary discharge water to selected aquatic organisms following procedures defined under the facility’s National Pollutant Discharge Elimination System (NPDES) permit. The results of the biological testing are contained in this report.

Statistically significant biological response of the test organisms was not detected at or below the acute critical effluent concentration (ACEC) of 11% or the chronic critical effluent concentration (CCEC) of 3.4% (Table 1-1). The effluent sample does not exceed the defined permit requirements (Table 1-2).

**Table 1-1. Toxicity Test Results Summary**

Test		NOEL (%)	LOEL (%)	LC <sub>50</sub> /EC <sub>50</sub> (%)
Acute	<i>Americamysis bahia</i> 48-Hour Survival	100	>100	>100
	<i>Atherinops affinis</i> 96-Hour Survival	100	>100	>100
Chronic	<i>Dendraster excentricus</i> Proportion Survived	100	>100	>100
	<i>Dendraster excentricus</i> Proportion Normal	100	>100	>100
	<i>Atherinops affinis</i> 7-Day Survival	100	>100	>100
	<i>Atherinops affinis</i> 7-Day Biomass	100	>100	>100

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub>/EC<sub>50</sub> = Lethal/Effect Concentration to 50% of test population

**Table 1-2. Permit Compliance Results**

<b>Permit Requirement</b>	<i>The Permittee must: acute and chronic toxicity testing on final effluent once in the last summer (August 2023) and once in the last winter (February 2024) prior to submission of the application for permit renewal. The series of concentrations must include the CCEC and the ACEC. The CCEC equals 3.4% effluent. The ACEC equals 11% effluent.</i>
<b>Result</b>	No statistically significant reduction in survival or growth was detected at or below the acute critical effluent concentration (ACEC) of 11% effluent or the chronic critical effluent concentration (CCEC) of 3.4% effluent for all tests conducted.

## 2. METHODS

The samples were analyzed for toxicity using criteria outlined in the Washington Department of Ecology’s (WDOE) Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria (WDOE WQ-R-95-80). These criteria are further defined through the Environmental Protection Agency’s (EPA) most recently promulgated effluent guidance documents outlined in Section 4.

To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

### 2.1 Sample Collection and Storage

Alon Asphalt Company personnel collected three grab samples on August 21, 23, and 25, 2023. The samples were transported by courier and were received at the laboratory on the same day as collection.

Sample temperatures upon receipt ranged from 0.1 to 2.1°C. Additional sample conditions are summarized in Table 2-1. The samples were held in a walk-in cold room at 4 ± 2°C in the dark until utilized for testing.

**Table 2-1. Sample Conditions upon Receipt**

Sample	Outfall #1-Grab		
Laboratory ID	P230821.01	P230823.01	P230825.01
Date/Time sampled	8/21/23; 0000	8/23/23; 0030	8/25/23; 0030
Date/Time received	8/21/23; 1305	8/23/23; 1117	8/25/23; 1250
Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	10.1	10.3	9.6
Temperature (°C) Upon Receipt Ideal: 4°C or <1 hour from sample: 0 – 20°C <4 hours from sample: 0 – 12°C ≥4 hours from sample: 0 – 6°C	2.1	1.0	0.1
pH (units) Recommended: 6 – 9	7.3	6.7	7.5
Salinity (ppt)	0.072	0.116	0.169
Conductivity (mS/cm)	215	297	256
Total Free Chlorine (mg/L)	0.02	0.03	0.01
Total Ammonia (mg/L)	0.0704	0.00	0.00

## 2.2 Bioassay Testing

Bioassay testing for this project consisted of two acute and two chronic bioassays. The tests conducted in support of this project are summarized in Table 2-2.

**Table 2-2. Biological Testing Performed**

Test Type	Test Descriptor	Species	Method
Acute	48-Hour Survival	<i>Americamysis bahia</i> (Opossum Shrimp)	WDOE WQ-R-95-80; EPA-821-R-02-012; Adapted from Test Method 2007.0; SOP TOX015.11
	96-Hour Survival	<i>Atherinops affinis</i> (Topsmelt)	WDOE WQ-R-95-80; EPA-821-R-02-012 Adapted from Test Method 2006.0 <sup>1</sup> ; SOP TOX001.12
Chronic	Echinoderm Survival and Development	<i>Dendraster excentricus</i> (Sand Dollar)	WDOE WQ-R-95-80, EPA 600/R-95/136; SOP TOX043.11
	7-Day Survival and Growth	<i>Atherinops affinis</i> (Topsmelt)	WDOE WQ-R-95-80; EPA 600/R-95/136 Test Method 1006.0; SOP TOX002.10

<sup>1</sup>Test Conditions for the acute *A. affinis* test are detailed in the “SUPPLEMENTAL LIST OF ACUTE TOXICITY TEST SPECIES” (EPA 2002). A specific test method for this analysis does not exist.

## 2.3 Organisms for Testing

*Americamysis bahia* and *Atherinops affinis* (Topsmelt) were purchased from Aquatic BioSystems Inc. (ABS) in Fort Collins, Colorado. ABS is a commercial supplier of test organisms that are used routinely for toxicity testing. Water quality measurements were collected from transport containers and the overall health of the organisms was visually confirmed by a laboratory technician.

Adult sand dollars (*Dendraster excentricus*) were collected by EcoAnalysts personnel on August 10, 2023. They were maintained under ambient seawater flow-through conditions at  $16 \pm 2^\circ\text{C}$  until utilized for testing. The overall health of the organisms was visually confirmed by a laboratory technician.

#### 2.4 Water for Bioassay Testing

Seawater diluent used in this study came from the northern Hood Canal at Port Gamble, Washington. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation of contaminants from this water supply. Chemical analysis of this water source is conducted and reviewed on an annual basis.

#### 2.5 Sample Adjustment

Salinity adjustments were necessary to bring the samples within the recommended test salinity for each marine test species. The effluent discharge samples arrived at salinities of 0.072 – 0.169 ppt. The salinity of the effluent samples was adjusted to the desired test salinity with Crystal Sea® MarineMix bioassay grade artificial salt. Table 2-3 summarizes the salinity adjustments performed on the project samples to create a salinity range within the tolerance limits of test species.

An artificial salt control sample was created to evaluate any potential negative impacts to the test organisms from the salinity adjustment alone. This sample was designated “Salt Control” and the results are discussed in Section 3.

**Table 2-3. Salinity Adjustment of Project Samples**

Sample ID: OUTFALL #1-GRAB	Sample Salinity Adjustment (ppt)
Sample 1: Collected 8/21/23	$30 \pm 2$
Sample 2: Collected 8/23/23	$30 \pm 2$
Sample 3: Collected 8/25/23	$30 \pm 2$

#### 2.6 Data Management and Analysis

Endpoint data was calculated for each replicate, and the mean value and standard deviation were determined for each sample concentration. All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting was reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance (EPA 2002 & EPA 1995). Statistical comparisons were performed using CETIS™ software.

#### 2.7 Quality Assurance/Quality Control

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the method specific guidance documents and the laboratory’s quality manual (QM). These objectives for accuracy and precision involve all aspects of the testing process, including the following:

- Source and Condition of Test Organisms
- Condition of Equipment
- Test Conditions
- Instrument Calibration
- Use of Reference Toxicants
- Record Keeping
- Data Evaluation

The batch of test organisms obtained was evaluated in a reference toxicant test that was run concurrently with the test period to establish the sensitivity of the test organisms. The reference toxicant LC<sub>50</sub> or EC<sub>50</sub> should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Practices (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced as a result of these analyses were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

### 3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A. Chain-of-custody and sample receipt logs are provided in Appendix B.

#### 3.1 Mysid (*Americamysis bahia*) Acute Test Results

The acute toxicity test with *A. bahia* was initiated on August 21, 2023, and was validated by 97.5% survival in the laboratory control, meeting the test acceptability criterion of ≥90% mean survival. Mean survival for all treatments is summarized in Table 3-1. The test conditions are summarized in Table 3-2.

Concentrations of 3.4, 11, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the ACEC of 11% effluent. Sample 1 (received 8/21/23) was used for test initiation and renewal.

Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static renewal test.

There was no significant difference observed between the laboratory control and the salt control indicating that artificial salts should not have contributed to any negative biological effects, if observed.

The EC<sub>50</sub> for the ammonia chloride reference-toxicant test was 32.5 mg/L total ammonia. These results were within two standard deviations of the laboratory mean for survival (Table 3-6). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-1. Endpoint Summary for *Americamysis bahia* Acute Test**

Sample ID	Conc. (%)	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC <sub>50</sub> Value (%)
Outfall #1-Grab	Control (0)	97.5	5.0	100	>100	>100
	Salt Control	100	0.0			
	3.4	100	0.0			
	11 <sup>1</sup>	97.5	5.0			
	25	95.0	5.8			
	50	97.5	5.0			
	100	100	0.0			

<sup>1</sup>Acute Critical Effluent Concentration (ACEC)  
NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level  
LC<sub>50</sub> = Lethal Concentration to 50% of test population

**Table 3-2. Test Condition Summary for Americamysis bahia Acute Test.**

<b>Test Duration / Type</b>	<b>48-Hour / Static Renewal</b>	
Species	<i>Americamysis bahia</i>	
Supplier	Aquatic BioSystems, Inc.	
Date acquired	8/18/23	
Test Dates	8/21/23 – 8/23/23	
Age at test initiation Recommended: 1 - 5 days	5 days	
Samples used:	Outfall #1-GRAB; P230821.01	
Holding Time at Initiation: Recommended: <36 hours	17 hours	
<b>Test Procedures</b>	WDOE WQ-R-95-80; EPA-821-R-02-012 Adapted from Test Method 2007.0; SOP TOX015.11	
Test location	EcoAnalysts, Port Gamble, WA	
Control water / Diluent	0.45 µm-filtered, North Hood Canal seawater	
Test Lighting	16-hour light / 8-hour dark	
Test Chamber	12 oz. cup	
Exposure volume	250 mL	
Replicates/treatment	4	
Concentration/treatment	3.4, 11, 25, 50, and 100%	
Organisms/replicate	10	
Feeding	Twice daily: 0.1 mL <i>Artemia</i> nauplii concentrate	
Test solution renewal	24-hours	
<b>Test Water Quality</b>		
Test Dissolved Oxygen	Recommended: > 4.0 mg/L	Actual: 6.0 – 9.2 mg/L
Test Temperature	Recommended: 20 ± 1°C	Actual: 19.0 – 21.0 °C
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 30 – 31 ppt
Test pH	Recommended: 6 - 9 units	Actual: 7.7 – 8.3 units
<b>Quality Assurance</b>		
Control performance standard	Recommended: ≥ 90% survival	Actual: 97.5%, meets acceptability criterion
Power Standard	Recommended: ≤29% (survival)	0%; meets criterion
Reference Toxicant	Total Ammonia	
Reference Toxicant Date	7/24/23	
Reference Toxicant LC <sub>50</sub>	32.5 mg/L	
Laboratory Mean LC <sub>50</sub>	41.3 mg/L	
Acceptable Range LC <sub>50</sub> (± 2 SD)	24.0 – 71.0 mg/L	
<b>Deviations from Test Protocol</b>	None	

### 3.2 *Dendraster excentricus* Test Results

The chronic toxicity test with *D. excentricus* conducted on sample “Outfall #1-Grab” was initiated on August 23, 2023, and was validated by 95.1% proportion normal and 3.9% Percent Minimum Significant Difference (PMSD) in the laboratory control, meeting the test acceptability criteria of ≥80% normal development and <25% PMSD. Mean survival and proportion normal are summarized in Table 3-3. The test conditions are summarized in Table 3-4.

Concentrations of 3.4, 11, 25, 50, and 100% effluent were prepared utilizing laboratory water. Water quality parameters were within the acceptable limits throughout the duration of the 72-hour static test.

There was no significant difference observed between the laboratory control and the salt control indicating that artificial salts should not have contributed to any negative biological effects, if observed.

The EC<sub>50</sub> for the copper chloride reference-toxicant test was 11.1 µg/L, which is within the two standard deviations of the laboratory mean (Table 3-4) at the time of testing. This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts Laboratory.

**Table 3-3 Results Summary for *Dendraster excentricus* Survival and Development Test**

Conc. (%)	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC <sub>50</sub> Value (%)
0	98.5	2.1	100	>100	>100
Salt Control	100	0.0			
3.4 <sup>2</sup>	97.5	5.0			
11 <sup>1</sup>	100	0.0			
25	100	0.0			
50	99.9	0.3			
100	100	0.0			
Conc. (%)	Mean Proportion Normal (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC <sub>50</sub> Value (%)
0	95.1	1.7	100	>100	>100
Salt Control	97.8	0.2			
3.4 <sup>2</sup>	96.4	2.8			
11 <sup>1</sup>	95.1	1.7			
25	96.4	1.5			
50	96.6	1.2			
100	96.7	0.5			

<sup>1</sup>Acute Critical Effluent Concentration (ACEC)

<sup>2</sup>Chronic Critical Effluent Concentration (CCEC)

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub> = Lethal Concentration to 50% of test population

EC<sub>50</sub> = Effect Concentration that caused a 50% reduction in normal development of test organisms

**Table 3-4. Test Condition Summary for *Dendroaster excentricus* Survival and Development Test.**

<b>Test Duration / Type</b>	<b>96-Hour; Static</b>	
Species	<i>Dendroaster excentricus</i>	
Supplier	Field Collected	
Date acquired	8/10/23	
Test Dates	8/23/23– 8/26/23	
Age at test initiation Recommended: <1-hour embryos	54 minutes	
Sample(s) used:	Outfall #1-Grab; P230823.01	
Holding Time at Initiation: Recommended: < 36 hours	15 hours	
<b>Test Procedures</b>	<b>WDOE WQ-R-95-80; EPA/600/R-95-136; SOP: TOX043.11</b>	
Test location	EcoAnalysts, Port Gamble, WA	
Control water / Diluent	0.45 µm-filtered, North Hood Canal seawater	
Test Lighting	16 hour light / 8 hour dark	
Test Chamber	30-mL Chamber	
Exposure volume	10 mL	
Organisms/replicate	Recommended: 250	Actual: 200
Replicates/treatment	4	
Concentration/treatment	3.4, 11, 25, 50 and 100%	
Feeding	None	
Test solution renewal	None	
<b>Test Water Quality</b>		
Test Dissolved Oxygen	Recommended: > 4.0 mg/L	Actual: 7.6 – 8.3 mg/L
Test Temperature	Recommended: 15 ± 1°C	Actual: 14.3 – 15.8 °C
Test pH	Recommended: 7– 9	Actual: 7.8 – 8.3
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 30 – 31 ppt
<b>Quality Assurance</b>		
Control performance standard (Normal development, PMSD)	Recommended: ≥80%, <25% PMSD	Actual: 95.1%, 3.9%; Pass
Zero-Time Coefficient of Variation	Recommended: ≤ 15%	Actual: 4.7%; Pass
Power Standard	Recommended: ≤ 39%	Actual: -1%; Pass
Reference Toxicant Date	8/23/23	
Reference Toxicant EC <sub>50</sub>	11.1 µg/L copper	
Laboratory Mean EC <sub>50</sub>	12.6 µg/L copper	
Acceptable Range EC <sub>50</sub> (± 2 sd)	8.23 – 19.2 µg/L copper	
<b>Deviations from Test Protocol</b>	None	

### 3.3 Topsmelt (*Atherinops affinis*) Acute Test Results

The acute toxicity test with *A. affinis* was initiated on August 21, 2023 and met the test acceptability criterion of  $\geq 90\%$  mean survival, with 95% survival in the laboratory control. Mean survival for all treatments is summarized in Table 3-5. The test conditions are summarized in Table 3-6.

Concentrations of 3.4, 11, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the ACEC of 11% effluent. Outfall #1-Grab sample one (P230821.01, received 8/21/23) was used for test initiation and test solution renewals on Day 2. Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static-renewal test.

There was no significant difference observed between the laboratory control and the salt control indicating that artificial salts should not have contributed to any negative biological effects, if observed.

The LC<sub>50</sub> for the copper chloride reference-toxicant test was 123.7  $\mu\text{g/L}$  copper and was within two standard deviations of the laboratory mean at the time of testing (Table 3-6). This indicates that the organisms were of similar sensitivity to others cultured by the supplier.

**Table 3-5. Endpoint Summary for *Atherinops affinis* Acute Test**

Sample ID	Conc. (%)	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC <sub>50</sub> Value (%)
Outfall #1-Grab	Control (0)	95.0	10.0	100	>100	>100
	Salt Control	92.5	5.0			
	3.4	95.0	5.8			
	11 <sup>1</sup>	100	0.0			
	25	100	0.0			
	50	95.0	10.0			
	100	97.5	5.0			

<sup>1</sup>Acute Critical Effluent Concentration (ACEC)

NOEL = No Observed Effect Level; LOEL = Lowest Observed Effect Level

LC<sub>50</sub> = Lethal Concentration to 50% of test population

**Table 3-6. Test Condition Summary for *Atherinops affinis* Acute Test**

Test Duration / Type	96-Hour / Static-Renewal	
Species	<i>Atherinops affinis</i>	
Supplier	Aquatic BioSystems, Inc.	
Date acquired	8/18/23	
Test Dates	8/21/23 – 8/25/23	
Age at test initiation Recommended: 7-15 days	11 days	
Samples used:	Outfall #1-Grab; P230821.01	
Holding Time at Initiation: Recommended: <36 hours	17 hours	
<b>Test Procedures</b>	WDOE WQ-R-95-80; EPA-821-R-02-012 Adapted from Test Method 2006.0; SOP TOX001.12	
Test location	EcoAnalysts, Port Gamble, WA	
Control water / Diluent	0.45 µm-filtered, North Hood Canal seawater	
Test Lighting	16-hour light / 8-hour dark	
Test Chamber	20 oz. cup	
Exposure volume	250 mL	
Replicates/treatment	4	
Concentration/treatment	3.4, 11, 25, 50, and 100%	
Organisms/replicate	10	
Feeding	Once daily: 0.1 mL <i>Artemia</i> nauplii concentrate	
Test solution renewal	Day 2	
<b>Test Water Quality</b>		
Test Dissolved Oxygen	Recommended: > 4.0 mg/L	Actual: 6.2 – 8.7 mg/L
Test Temperature	Recommended: 20 ± 1°C	Actual: 19.0 – 20.5 °C
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 30 – 31 ppt
Test pH	Recommended: 6 - 9 units	Actual: 7.6 – 8.3 units
<b>Quality Assurance</b>		
Control performance standards	Recommended: ≥ 90% survival	Actual: 95%, meets acceptability criterion
	Power Standard: ≤29% (survival)	-5%; meets criterion
<b>Reference Toxicant</b>	<b>Copper Chloride</b>	
Reference Toxicant LC <sub>50</sub>	123.7 µg/L	
Supplier Mean LC <sub>50</sub>	164.2 µg/L	
Acceptable Range LC <sub>50</sub> (± 2 sd)	80.4 – 336 µg/L	
<b>Deviations from Test Protocol</b>	None	

### 3.4 Topsmelt (*Atherinops affinis*) Chronic Test Results

The chronic toxicity test with *A. affinis* was initiated on August 21, 2023. The test was validated by 92% mean control survival, meeting the control acceptability criterion of  $\geq 80\%$  mean survival. The control treatment had a mean dry weight of 0.930 mg and a mean dry biomass of 0.854 mg, meeting the recommended growth criterion of  $\geq 0.850$  mg mean dry weight.

Mean survival and growth endpoints are summarized in Table 3-7. The statistical results are summarized in Table 3-8 and the test conditions are summarized in Table 3-9.

Concentrations of 3.4, 11, 25, 50, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the CCEC of 3.4% effluent. The initial sample (received 8/21/23) was used for test initiation and Days 1 and 2 test solution renewals while the second and third samples (received 8/23/23 and 8/25/23) were used for the remaining test solution renewals. Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test.

There was no significant difference observed between the laboratory control and the salt control for any of the endpoints tested, indicating that artificial salts should not have contributed to any negative biological effects, if observed.

The LC<sub>50</sub> for the copper chloride reference-toxicant test was 77.5  $\mu\text{g Cu/L}$  for survival and 67.7  $\mu\text{g Cu/L}$  for mean dry biomass. These results were within two standard deviations of the laboratory mean for survival and mean dry biomass (Table 3-9). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-7. Endpoint Summary for the *Atherinops affinis* Chronic Test**

Conc. (%)	Outfall #1-Grab		
	Mean Survival (%)	Mean Growth (mg) <sup>1</sup>	Mean Biomass (mg) <sup>2</sup>
Control (0)	92	0.930	0.854
Salt Control	88	1.170	1.019
3.4 <sup>3</sup>	96	1.049	1.014
11 <sup>4</sup>	100	0.972	0.972
25	92	0.871	0.809
50	100	0.898	0.898
100	100	0.999	0.999

<sup>1</sup> Average weight (mg) per survivor.

<sup>2</sup> Average weight (mg) per original number of animals stocked (Biomass).

<sup>3</sup> Chronic Critical Effluent Concentration (CCEC).

<sup>4</sup> Acute Critical Effluent Concentration (ACEC).

**Table 3-8. Statistical Results Summary for *Atherinops affinis* Chronic Test**

Endpoint	OUTFALL #1-GRAB		
	Survival	Dry Weight	Biomass
NOEL (%)	100	100	100
LOEL (%)	>100	>100	>100
LC <sub>50</sub> / EC <sub>50</sub> (%)	>100	>100	>100

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub>/EC<sub>50</sub> = Lethal/Effect Concentration to 50% of test population

**Table 3-9. Test Condition Summary for *Atherinops affinis* Chronic Test**

Test Duration / Type		7-Day / Static-Renewal	
Species		<i>Atherinops affinis</i>	
Supplier		Aquatic Bio Systems, Inc.	
Date acquired		8/18/23	
Test Dates		8/21/23 – 8/28/23	
Age at test initiation (Recommended: 9-15 days)		11 Days	
Samples used:		Outfall #1-Grab; P230821.01, P230823.01, P230825.01	
Sample Holding Time at Initiation: Recommended: <36 hours		17 hours	
<b>Test Procedures</b>		WDOE WQ-R-95-80; EPA 600/R-95/136 Test Method 1006.0; SOP TOX002.10	
Test location		EcoAnalysts; Port Gamble, WA	
Control water / Diluent		0.45 µm-filtered, North Hood Canal seawater	
Test Lighting		16-hour light / 8-hour dark	
Test Chamber		20 oz. cup	
Exposure volume		250 mL	
Replicates/treatment		5	
Concentration/treatment		3.4, 11, 25, 50 and 100%	
Organisms/replicate		5	
Feeding		250 Artemia nauplii am / 500 pm, except Day 7	
Test solution renewal		Daily (Days 1-6)	
<b>Test Water Quality</b>			
Test Dissolved Oxygen		Recommended: > 4.0 mg/L	5.7 – 8.8 mg/L
Test Temperature		Recommended: 20 ± 1°C	18.5 – 21.3 °C
Test Salinity		Recommended: 30 ± 2 ppt	28 – 31 ppt
Test pH		Recommended: 6 - 9 units	7.6 – 8.3
<b>Quality Assurance</b>			
Control performance standards		Survival Recommended: ≥ 80%	Actual: 92%; meets acceptability criterion
		Growth Recommended: ≥ 0.850 mg	Actual: 0.930 mg, meets acceptability criterion
		Power Standard: ≤39% (Growth)	-13%; meets criterion
<b>Reference Toxicant</b>		<b>Copper Chloride</b>	
Survival	Reference Toxicant LC <sub>50</sub> (must be < 205 µg/L)	77.5 µg Cu/L	
	Laboratory Mean LC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD)	110.4 µg Cu/L (69.3 – 176 µg Cu/L)	
	PMSD (must be <25%)	34.4%	
Biomass	Reference Toxicant EC <sub>50</sub>	67.7 µg Cu/L	
	Laboratory Mean EC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD)	107.7 µg Cu/L (66.7 – 174 µg Cu/L)	
	PMSD (must be <50%)	29.9%	
<b>Deviations from Test Protocol</b>		None	

## 4. REFERENCES

- CETIS. 2022. CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.
- Lee, D.R. 1980. Reference toxicants in quality control of aquatic bioassays: Aquatic invertebrate bioassays. In Buikema AL Jr, Cairns J Jr, eds, *Proceedings*, 2nd Annual Symposium on Aquatic Toxicology. STP 715. American Society for Testing and Materials, Philadelphia, PA, pp 188–199.
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, First Edition. EPA/600/R-95-136.
- USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA-821-R-02-012.
- WDOE. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

## APPENDIX A

Statistical Comparisons

Laboratory Documents

**A1: Outfall #1-Grab**

A1.1: *A. bahia* Acute

A1.2: *D. excentricus* Chronic

A1.3: *A. affinis* Acute

A1.4: *A. affinis* Chronic

**A2: Reference Toxicant**

A2.1: *A. bahia* Acute

A2.2: *D. excentricus* Chronic

A2.3: *A. affinis* Acute

A2.4: *A. affinis* Chronic

## **APPENDIX A1.1**

Outfall #1-Grab

*Americamysis bahia* (Opossum Shrimp)

48-Hour Acute Test

**CETIS Summary Report**

Report Date: 21 Sep-23 15:43 (p 1 of 1)  
 Test Code/ID: P230821.01 A.b. / 04-8307-2011

**Mysidopsis 96-h Acute Survival Test**

EcoAnalysts

Batch ID: 10-4053-1108	Test Type: Survival (48h)	Analyst:
Start Date: 21 Aug-23 16:53	Protocol: EPA/821/R-02-012 (2002)	Diluent: Laboratory Seawater
Ending Date: 23 Aug-23 15:16	Species: Americamysis bahia	Brine: Crystal Sea Marine Mix
Test Length: 46h	Taxon: Malacostraca	Source: Aquatic Biosystems, CO Age: 5d
Sample ID: 12-7990-1654	Code: P230821.01	Project: Alon Asphalt Company
Sample Date: 21 Aug-23	Material: Industrial Effluent	Source: Alon Asphalt (WA0003239)
Receipt Date: 21 Aug-23 13:05	CAS (PC):	Station: Outfall #1 - Grab
Sample Age: 17h (2.1 °C)	Client: Alon Asphalt	

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
08-4604-1580	48h Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	7.43%	1	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
16-9404-8408	48h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			EC25	>100	---	---	<1	
			EC40	>100	---	---	<1	
			EC50	>100	---	---	<1	

**48h Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
0	SC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
3.4		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%
11		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
25		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	2.56%
50		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	0.00%
100		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-2.56%

**48h Proportion Survived Detail**

MD5: DEF1389B17B925A8041EADFE38D42CED

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	0.9000	1.0000	1.0000
0	SC	1.0000	1.0000	1.0000	1.0000
3.4		1.0000	1.0000	1.0000	1.0000
11		0.9000	1.0000	1.0000	1.0000
25		1.0000	0.9000	0.9000	1.0000
50		1.0000	0.9000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000

**48h Proportion Survived Binomials**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	9/10	10/10	10/10
0	SC	10/10	10/10	10/10	10/10
3.4		10/10	10/10	10/10	10/10
11		9/10	10/10	10/10	10/10
25		10/10	9/10	9/10	10/10
50		10/10	9/10	10/10	10/10
100		10/10	10/10	10/10	10/10

**CETIS Analytical Report**

Report Date: 21 Sep-23 15:48 (p 1 of 2)  
 Test Code/ID: P230821.01 A.b. / 04-8307-2011

**Mysidopsis 96-h Acute Survival Test**

EcoAnalysts

<b>Analysis ID:</b> 13-0612-6495	<b>Endpoint:</b> 48h Proportion Survived	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 21 Sep-23 15:34	<b>Analysis:</b> Nonparametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 21 Sep-23 15:24	<b>MD5 Hash:</b> A8CD157DF76DF82BDB4AE3121BCF49B	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 10-4053-1108	<b>Test Type:</b> Survival (48h)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:53	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 23 Aug-23 15:16	<b>Species:</b> Americamysis bahia	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 46h	<b>Taxon:</b> Malacostraca	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 5d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed 48h proportion survived endpoint	5.20%

**Wilcoxon Rank Sum Two-Sample Test**

Control I	vs	Control II	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	20	---	1	Exact	1.0000	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0033199	0.0033199	1	1	0.3559	Non-Significant Effect
Error	0.0199195	0.0033199	6			
Total	0.0232394		7			

**ANOVA Assumptions Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	9	13.75	0.0240	Equal Variances
	Mod Levene Equality of Variance Test	1	13.75	0.3559	Equal Variances
	Variance Ratio F Test				Indeterminate
Distribution	Anderson-Darling A2 Test	1.162	3.878	0.0049	Non-Normal Distribution
	Kolmogorov-Smirnov D Test	0.375	0.3313	0.0015	Non-Normal Distribution
	Shapiro-Wilk W Normality Test	0.7065	0.6451	0.0027	Non-Normal Distribution

**48h Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9750	0.8954	1.0000	1.0000	0.9000	1.0000	0.0250	5.13%	0.00%
0	SC	4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-2.56%

**Angular (Corrected) Transformed Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3710	1.2420	1.5010	1.4120	1.2490	1.4120	0.0407	5.94%	0.00%
0	SC	4	1.4120	1.4120	1.4120	1.4120	1.4120	1.4120	0.0000	0.00%	-2.97%

**48h Proportion Survived Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	0.9000	1.0000	1.0000
0	SC	1.0000	1.0000	1.0000	1.0000

**Angular (Corrected) Transformed Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.4120	1.2490	1.4120	1.4120
0	SC	1.4120	1.4120	1.4120	1.4120





Client	Alon Asphalt Company
Project	Alon Asphalt Company
Project Number	PG1801
Project Manager	M. Seibert
Date Sample Received	8/21/2023
Test type	48-Hour Acute Toxicity with Mysid
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	08/21/23
Test Species	Americamysis bahia
Organism Batch	ABS081823.02
Organism Acquired	8/18/2023
Organism Acclimation	3
Organism Age	5 days
Test Protocol	TOX 015
Test Location	Bath 1
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	10
Test Chamber Size	12 oz
Exposure Volume	250 mL
Feeding Information	0.1 mL of Artemia nauplii/chamber twice per day
Test Dissolved Oxygen	> 4.0
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	19	21
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 11653 JL MS  
 TEST END TIME/INIT: 1516 49

CLIENT SAMPLE ID	LAB ID
Outfall #1- Grab	P230821.01

Concentrations	
1	Control
2	Salt Control
3	3.4%
4	11%
5	25%
6	50%
7	100%
8	.
9	.

Food Batch ID
281729.00

CSMM Batch #
A2017620

Treatment	Rep	Chamber
Control	1	26
Control	2	8
Control	3	5
Control	4	7
Salt Control	1	27
Salt Control	2	28
Salt Control	3	19
Salt Control	4	20
3.4%	1	4
3.4%	2	14
3.4%	3	24
3.4%	4	12
11%	1	17
11%	2	9
11%	3	18
11%	4	6
25%	1	15
25%	2	22
25%	3	16
25%	4	2
50%	1	10
50%	2	13
50%	3	11
50%	4	1
100%	1	21
100%	2	23
100%	3	25
100%	4	3
.	1	
.	2	
.	3	
.	4	
.	1	
.	2	
.	3	
.	4	

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 015
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/23/23	SPECIES	Americamysis bahia
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

48-Hour Acute Toxicity with Mysid

	Concentration (%)	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
		> 4.0	19 - 21	28 - 32	6 - 9
<b>Day 0</b>	Control	7.6	19.2	31	8.0
Stock	Salt Control	7.3	19.0	30	8.3
Date 8/21/23	3.4%	7.7	19.3	31	8.0
Time 1545	11%	7.7	19.1	31	8.1
Tech TW	25%	7.8	19.4	31	8.0
Meter # 7	50%	8.3	20.4	30	8.0
Feed: LG	100%	9.2	20.8	30	7.9
<b>Day 1</b>	Control	6.0	20.0	31	7.9
Rep 1	Salt Control	6.8	19.9	30	8.2
Date 8/22/23	3.4%	7.0	19.9	31	8.0
Time 1050	11%	6.9	20.0	31	8.0
Tech LG	25%	6.8	20.0	30	8.0
Meter # 9	50%	6.7	20.1	30	8.1
	100%	6.5	20.0	30	8.1
<b>Day 1</b>	Control	7.4	19.5	31	8.1
Renewal Stock	Salt Control	7.6	19.8	30	8.3
Date 8/22/23	3.4%	7.5	19.5	31	8.1
Time 1106	11%	7.6	20.3	31	8.1
Tech LG	25%	7.7	20.5	31	8.1
Meter # 9	50%	8.0	21.0	30	8.1
	100%	8.6	20.8	30	8.0
<b>Day 2</b>	Control	6.5	20.3	31	7.7
Rep 2	Salt Control	6.4	20.2	31	8.1
Date 8/23/23	3.4%	6.2	20.1	31	7.9
Time 0858	11%	① <del>6.5</del> 6.0	20.2	31	7.9
Tech JI	25%	6.5	20.2	31	7.9
Meter # 8	50%	6.1	20.2	31	8.0
	100%	6.7	20.3	30	8.1

① I.E, TW, 8/21/23, JI 8/23/23

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 015
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/23/23	SPECIES	Americamysis bahia
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

**Abbreviation Key:**

- NB = No Body
- FB = Found Body
- ST = Stranded

**48-Hour Acute Toxicity with Mysid**

Concentration (%)	REP	Day 1		Day 2	
		Alive	Dead	Alive	Dead
		Date	8/22/23	Date	8/23/23
		Time	1322	Time	1516
		Tech	UG	Tech	UG
Control	1	10	0	10	0
	2	10	0	9	1 NB
	3	10	0	10	0
	4	10	0	10	0
Salt Control	1	10	0	10	0
	2	10	0	10	0
	3	10	0	10	0
	4	10	0	10	0
3.4%	1	10	0	10	0
	2	10	0	10	0
	3	10	0	10	0
	4	10	0	10	0
11%	1	9	1	9	0
	2	10	0	10	0
	3	10	0	10	0
	4	10	0	10	0
25%	1	10	0	10	0
	2	10	0	9	1 NB
	3	9	1	9	0
	4	10	0	10	0
50%	1	10	0	10	0
	2	9	1	9	0
	3	10	0	10	0
	4	10	0	10	0
100%	1	10	0	10	0
	2	10	0	10	0
	3	10	0	10	0
	4	10	0	10	0

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 015
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/23/23	SPECIES	<i>Americamysis bahia</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

**Abbreviation Key:**

- NB = No Body
- FB = Found Body
- ST = Stranded

**48-Hour Acute Toxicity with Mysid**

		Day 1		Day 2	
		Date	8/22/23	Date	8/23/23
		Time	1322	Time	1516
		Tech	LG	Tech	SI
Concentration (%)	REP.	Alive	Dead	Alive	Dead
Feed (Init.)	AM	TW			
0.1 mL of Artemia nauplii/chamber twice per day	PM	TW			

v.2

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 015
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/23/23	SPECIES	Americamysis bahia
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

48-Hour Acute Toxicity with Mysid

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	1000.0	1000	FSW	
	Salt Control	#VALUE!	#VALUE!	1000		
	3.4%	34	966.0	1000		
	11%	110	890.0	1000		
	25%	250	750.0	1000		
	50%	500	500.0	1000		
	100%	1000	0.0	1000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1	0%	0	800.0	800
	Salt Control	#VALUE!	#VALUE!	800
	3.4%	27.2	772.8	800
	11%	88	712.0	800
	25%	200	600.0	800
	50%	400	400.0	800
	100%	800	0.0	800

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
8/21/23	7	P230821.01	FSW081523.01	LG
8/24/23	7	P230821.01	FSW081323.01	LG

## POWER STANDARD CALCULATIONS

*Americamysis bahia* Acute Survival

Acute Power Standard Calculation

Replicate	Number Surviving				Mean
	1	2	3	4	
ACEC (11)	9	10	10	10	9.75
Control	10	9	10	10	9.75

Control Mean - ACEC Mean

0

Difference Divided by Control Mean

0

Express as %

0%

≤29% meets the power standard

Pass

## ORGANISM RECEIPT LOG

<b>Date:</b> 8/18/23		<b>Time:</b> 1230		<b>Batch No.</b> ABS081823.02			
<b>Organism:</b> Americamysis bahia							
<b>Source / Supplier:</b> Aquatic Biosystems							
<b>No. Ordered:</b> 310		<b>No. Received:</b> 340		<b>Source Batch:</b> Collection date, hatch date, etc.): Hatch. 8/16/23			
<b>Condition of Organisms:</b> Good				<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): 2 days			
<b>Shipper:</b> FedEx				<b>B of L (Tracking No.)</b> 137496024119			
<b>Condition of Container:</b> Good				<b>Received By:</b> M. Seibert			
Container	D.O. (mg/L)	Temp. (°C)	Cond. or (Sal.) (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	11.4	20.8	27 ppt	8.2	—	—	MS
<i>*if &gt;10% contact lab manager</i>							
<b>Notes:</b>							

1300 Blue Spruce Drive, Suite C  
Fort Collins, Colorado 80524



Toll Free: 800/331-5916  
Tel: 970/484-5091 Fax: 970/484-2514

### ORGANISM HISTORY

DATE: 8/17/2023

SPECIES: Americamysis bahia (formerly Mysidopsis)

AGE: 1 day

LIFE STAGE: Juvenile

HATCH DATE: 8/16/2023

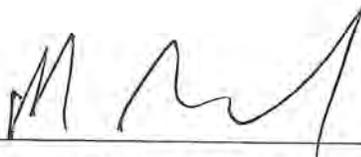
BEGAN FEEDING: Immediately

FOOD: Artemia sp.

### Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>26°C</u>	<u>24-26 °C</u>
SALINITY/CONDUCTIVITY:	<u>25 ppt</u>	<u>21-30 ppt</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>140 mg/l</u>	<u>140-175 mg/l</u>
pH:	<u>7.80</u>	<u>7.80-8.20</u>

### Comments:

  
\_\_\_\_\_  
Facility Supervisor

## **APPENDIX A1.2**

Outfall #1-Grab

*Dendraster excentricus* (Sand Dollar)

72-Hour Chronic Test

**CETIS Summary Report**

Report Date: 22 Sep-23 14:43 (p 1 of 2)  
 Test Code/ID: P230823.01 D.e. / 12-4316-1896

**Echinoid Embryo-Larval Development Test**

EcoAnalysts

<b>Batch ID:</b> 15-0836-5729	<b>Test Type:</b> Development-Survival	<b>Analyst:</b>
<b>Start Date:</b> 23 Aug-23 15:04	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 26 Aug-23 16:39	<b>Species:</b> Dendraster excentricus	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 74h	<b>Taxon:</b> Echinoidea	<b>Source:</b> In-House Culture
		<b>Age:</b> <1h
<b>Sample ID:</b> 09-2640-8618	<b>Code:</b> P230823.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 23 Aug-23 00:30	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 23 Aug-23 11:17	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 15h (1 °C)	<b>Client:</b> Alon Asphalt	

**Multiple Comparison Summary**

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
13-8299-2936	Proportion Normal	Dunnett Multiple Comparison Test	100	>100	---	3.88%	1	1
10-1335-1871	Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	3.24%	1	1

**Point Estimate Summary**

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
12-8473-2628	Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
07-3476-0965	Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	

**Proportion Normal Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9513	0.9240	0.9787	0.9319	0.9719	0.0086	0.0172	1.81%	0.00%
0	SC	4	0.9780	0.9753	0.9806	0.9764	0.9803	0.0008	0.0017	0.17%	-2.80%
3.4		4	0.9643	0.9195	1.0090	0.9265	0.9889	0.0141	0.0282	2.92%	-1.37%
11		4	0.9505	0.9236	0.9775	0.9358	0.9704	0.0085	0.0169	1.78%	0.08%
25		4	0.9636	0.9397	0.9875	0.9522	0.9838	0.0075	0.0150	1.56%	-1.29%
50		4	0.9656	0.9458	0.9853	0.9510	0.9799	0.0062	0.0124	1.28%	-1.49%
100		4	0.9666	0.9594	0.9738	0.9614	0.9724	0.0023	0.0045	0.47%	-1.60%

**Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9850	0.9512	1.0190	0.9550	1.0000	0.0106	0.0212	2.15%	0.00%
0	SC	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.52%
3.4		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	1.02%
11		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.52%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.52%
50		4	0.9988	0.9948	1.0030	0.9950	1.0000	0.0013	0.0025	0.25%	-1.40%
100		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.52%

MARH

# CETIS Summary Report

Report Date: 22 Sep-23 14:43 (p 2 of 2)  
 Test Code/ID: P230823.01 D.e. / 12-4316-1896

## Echinoid Embryo-Larval Development Test

EcoAnalysts

### Proportion Normal Detail

MD5: 8841B6B951865FDC3F7336D5FFD29B65

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9319	0.9442	0.9573	0.9719
0	SC	0.9803	0.9772	0.9780	0.9764
3.4		0.9596	0.9823	0.9265	0.9889
11		0.9358	0.9704	0.9372	0.9587
25		0.9522	0.9522	0.9838	0.9664
50		0.9510	0.9703	0.9610	0.9799
100		0.9724	0.9614	0.9657	0.9670

### Proportion Survived Detail

MD5: B019AEE7C12D810532116975016F29C8

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9550	0.9850	1.0000	1.0000
0	SC	1.0000	1.0000	1.0000	1.0000
3.4		1.0000	1.0000	1.0000	0.9000
11		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	0.9950
100		1.0000	1.0000	1.0000	1.0000

### Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	178/191	186/197	202/211	242/249
0	SC	199/203	214/219	222/227	207/212
3.4		214/223	222/226	189/204	178/180
11		204/218	197/203	209/223	209/218
25		199/209	199/209	243/247	230/238
50		194/204	196/202	222/231	195/199
100		211/217	199/207	197/204	205/212

### Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	191/200	197/200	200/200	200/200
0	SC	200/200	200/200	200/200	200/200
3.4		200/200	200/200	200/200	180/200
11		200/200	200/200	200/200	200/200
25		200/200	200/200	200/200	200/200
50		200/200	200/200	200/200	199/200
100		200/200	200/200	200/200	200/200



**CETIS Analytical Report**

Report Date: 22 Sep-23 14:30 (p 2 of 4)  
 Test Code/ID: P230823.01 D.e. / 12-4316-1896

**Echinoid Embryo-Larval Development Test**

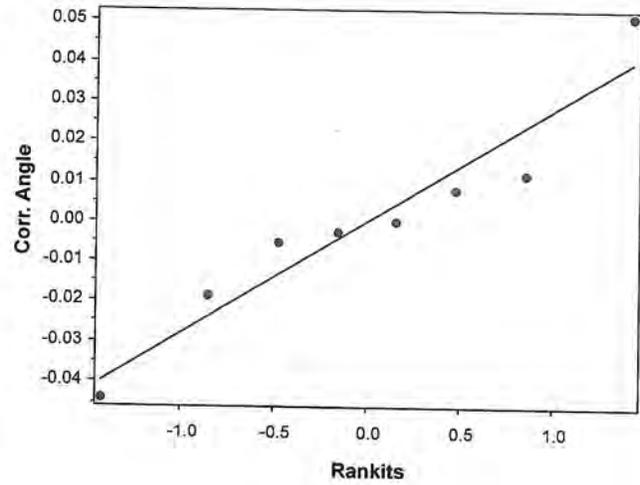
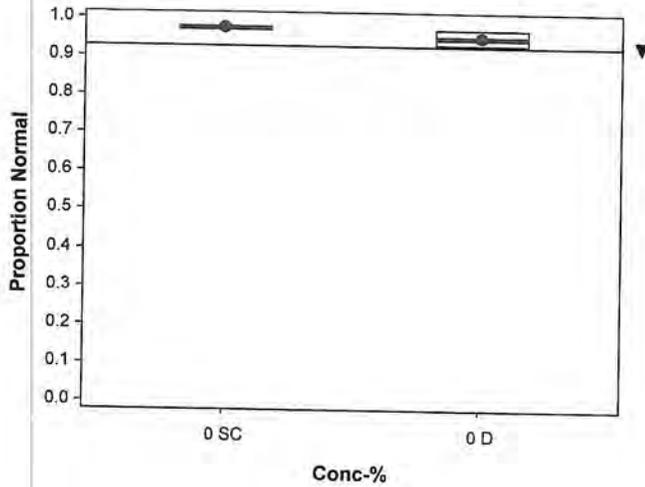
EcoAnalysts

Analysis ID: 08-5516-2682      Endpoint: Proportion Normal      CETIS Version: CETISv2.1.4  
 Analyzed: 22 Sep-23 14:18      Analysis: Parametric-Two Sample      Status Level: 1  
 Edit Date: 22 Sep-23 13:59      MD5 Hash: 26BD7B5BCF18224262A26DB2942FE561      Editor ID: 006-677-240-1

**Proportion Normal Binomials**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	178/191	186/197	202/211	242/249
0	SC	199/203	214/219	222/227	207/212

**Graphics**



**CETIS Analytical Report**

Report Date: 22 Sep-23 14:30 (p 3 of 4)  
 Test Code/ID: P230823.01 D.e. / 12-4316-1896

**Echinoid Embryo-Larval Development Test**

EcoAnalysts

<b>Analysis ID:</b> 13-6167-5911	<b>Endpoint:</b> Proportion Survived	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 22 Sep-23 14:18	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 22 Sep-23 13:59	<b>MD5 Hash:</b> FF5213224E24703038C966EB2EB78F1F	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 15-0836-5729	<b>Test Type:</b> Development-Survival	<b>Analyst:</b>
<b>Start Date:</b> 23 Aug-23 15:04	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 26 Aug-23 16:39	<b>Species:</b> Dendraster excentricus	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 74h	<b>Taxon:</b> Echinoidea	<b>Source:</b> In-House Culture <b>Age:</b> <1h
<b>Sample ID:</b> 09-2640-8618	<b>Code:</b> P230823.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 23 Aug-23 00:30	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 23 Aug-23 11:17	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 15h (1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed proportion survived endpoint	2.57%

**Unequal Variance t Two-Sample Test**

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	3	-1.559	2.353	0.1003	CDF	0.8916	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0088321	0.0088321	1	2.431	0.1700	Non-Significant Effect
Error	0.0218023	0.0036337	6			
Total	0.0306344		7			

**ANOVA Assumptions Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	12.81	13.75	0.0117	Equal Variances
	Mod Levene Equality of Variance Test	8.537	13.75	0.0266	Equal Variances
	Variance Ratio F Test				Indeterminate
Distribution	Anderson-Darling A2 Test	0.7428	3.878	0.0528	Normal Distribution
	Kolmogorov-Smirnov D Test	0.25	0.3313	0.1599	Normal Distribution
	Shapiro-Wilk W Normality Test	0.8432	0.6451	0.0813	Normal Distribution

**Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9850	0.9512	1.0000	0.9950	0.9550	1.0000	0.0106	2.15%	0.00%
0	SC	4	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	-1.52%

**Angular (Corrected) Transformed Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.4690	1.3330	1.6050	1.5060	1.3570	1.5350	0.0426	5.80%	0.00%
0	SC	4	1.5350	1.5350	1.5360	1.5350	1.5350	1.5350	0.0000	0.00%	-4.52%

**Proportion Survived Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9550	0.9850	1.0000	1.0000
0	SC	1.0000	1.0000	1.0000	1.0000

**Angular (Corrected) Transformed Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.3570	1.4480	1.5350	1.5350
0	SC	1.5350	1.5350	1.5350	1.5350



**CETIS Test Data Worksheet**

Report Date: 22 Sep-23 14:47 (p 1 of 1)  
 Test Code/ID: P230823.01 D.e. / 12-4316-1896

**Echinoid Embryo-Larval Development Test** EcoAnalysts

Start Date: 23 Aug-23 15:04 Species: Dendroaster excentricus Sample Code: P230823.01  
 End Date: 26 Aug-23 16:39 Protocol: EPA/600/R-95/136 (1995) Sample Source: Alon Asphalt  
 Sample Date: 23 Aug-23 00:30 Material: Industrial Effluent Sample Station: Outfall #1 - Grab

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	23	200	191	191	178	
0	D	2	18	200	197	197	186	
0	D	3	15	200	211	211	202	
0	D	4	6	200	249	249	242	
0	SC	1	21	200	203	203	199	
0	SC	2	24	200	219	219	214	
0	SC	3	16	200	227	227	222	
0	SC	4	3	200	212	212	207	
3.4		1	19	200	223	223	214	
3.4		2	7	200	226	226	222	
3.4		3	10	200	204	204	189	
3.4		4	22	200	180	180	178	
11		1	9	200	218	218	204	
11		2	1	200	203	203	197	
11		3	8	200	223	223	209	
11		4	14	200	218	218	209	
25		1	28	200	209	209	199	
25		2	2	200	209	209	199	
25		3	12	200	247	247	243	
25		4	20	200	238	238	230	
50		1	26	200	204	204	194	
50		2	27	200	202	202	196	
50		3	25	200	231	231	222	
50		4	13	200	199	199	195	
100		1	4	200	217	217	211	
100		2	11	200	207	207	199	
100		3	5	200	204	204	197	
100		4	17	200	212	212	205	

Version V.2

GENERAL

Client	Alon Asphalt Co.
Project	Alon Asphalt Co.
Project Number	PG1801
Project Manager	M. Seibert
Date Sample Received	8/23/2023
Test type	Chronic Toxicity Using Echinoderm Larvae
Matrix	Liquid
Test Acceptability	≥80% normal development in control, MSD <25%
Test Start Date	08/23/23
Test Species	Dendraster excentricus
Organism Batch	DE081023.01
Organism Acquired	8/10/2023
Organism Acclimation	13
Organism Age	Various Adult
Test Protocol	TOX 043 - WDOE
Test Location	Incubator 1
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	250
Test Chamber Size	26 mL
Exposure Volume	10 mL
Test Dissolved Oxygen	> 4.0
Test Temperature	15 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	14	16
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 1504 JL, J1, LG  
 TEST END TIME/INIT: 1639 NL 8/26/23

CLIENT SAMPLE ID	LAB ID
Outfall #1- Grab	P230823.01

Concentrations	
1	Control
2	Salt Control
3	3.4%
4	11%
5	25%
6	50%
7	100%
8	-
9	-

Salinity Adjustment CSMM Batch #
A2017620

Formalin Lot #
220304-50

Rose Bangel Batch #
5135

Chronic Echinoderm WET Test

v.2

CLIENT	Alon Asphalt Co.	DATE RECEIVED	8/23/23	PROTOCOL	TOX 043 - WDOE
PROJECT	Alon Asphalt Co.	TEST START DATE	8/23/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE		SPECIES	<i>Dendraster excentricus</i>
LAB SAMPLE ID	P230823.01	MATRIX	Liquid	NO. OF ORGANISMS	250

Chronic Toxicity Using Echinoderm Larvae

SPAWNING METHOD		INITIAL SPAWNING TIME		FINAL SPAWNING TIME	
KCI		1325		1405	
MALES	FEMALES	SPERM VIABILITY		EGG CONDITION	
4	3	Good		Good	
BEGIN FERTILIZATION		END FERTILIZATION		CONDITION OF EMBRYOS	
1410		1504		Good	

TIME OF INITIATION	INITIALS
15:04	JL, JL, LG

EMBRYO DENSITY CALCULATIONS

# of embryos in 1 mL of 100X diluted embryo stock			# embryos in original stock = # of embryos in diluted stock x 100	
Count 1	Count 2	Mean		
32	37	34.5	3450	
Percentage of embryo stock needed = 2700 embryos per 1 mL/# embryos in original stock				
0.78				
mL of egg stock to add to FSW to achieve total volume = percentage of embro stock needed * 100 mL (or desired volume of embryo stock)				
78.26 Add this volume to beaker and dilute to 40 mL (or desired volume of embryo stock) with FSW = final embryo stock				
Add 0.1 mL of final embryo stock to test chambers				

v.2

CLIENT	Alon Asphalt Co.	DATE RECEIVED	8/23/23	PROTOCOL	TOX 043 - WDOE
PROJECT	Alon Asphalt Co.	TEST START DATE	8/23/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/26/23	SPECIES	<i>Dendraster excentricus</i>
LAB SAMPLE ID	P230823.01	MATRIX	Liquid	NO. OF ORGANISMS	250

**Chronic Toxicity Using Echinoderm Larvae**

	Concentration (%)	DO (mg/L) > 4.0	TEMP (°C) 14 - 16	SALINITY (ppt) 28 - 32	pH 6 - 9
<b>Day 0</b>	Control	7.6	① 18.8 ② 15.8	31	7.8
Stock	Salt Control	7.6	② 15.8	30	8.3
Date 8/23/23	3.4%	7.6	② 15.8	31	8.0
Time 14:40	11%	7.7	② 15.8	31	8.0
Tech J1	25%	7.9	② 15.8	31	8.0
Meter # 8	50%	7.8	② 15.8	31	8.1
	100%	8.0	② 15.8	31	8.2
<b>Day 1</b>	Control		② 14.3		
Surrogate	Salt Control		② 14.3		
Date 8/24/23	3.4%		② 14.3		
Time 1320	11%		② 14.3		
Tech LG	25%		② 14.3		
Meter # T16	50%		② 14.3		
	100%		② 14.3		
<b>Day 2</b>	Control		② 14.3		
Surrogate	Salt Control		② 14.3		
Date 8/25/23	3.4%		② 14.3		
Time 0900	11%		② 14.3		
Tech NL	25%		② 14.3		
Meter # T21	50%		② 14.3		
*only measure DO, temp, salinity if last day					
<b>Day 3</b>	Control	7.9	② 14.5	31	7.8
Surrogate	Salt Control	8.1	② 14.5	31	8.1
Date 8/26/23	3.4%	8.2	② 14.5	31	8.0
Time 1637	11%	8.3	② 14.5	31	8.0
Tech NL	25%	8.3	② 14.5	31	8.1
Meter # 8	50%	8.3	② 14.5	31	8.2
*only measure DO, temp, salinity if last day					
<b>Day 4</b>	Control		② 14.5		
Surrogate	Salt Control				
Date	3.4%				
Time	11%				
Tech	25%				
Meter #	50%				
	100%				

① 15 J1 8/23/23, NL 8/26

② Temp taken from temp blank J1 8/23/23, LG 8/24/23, NL 8/25/23, NL 8/26

v.2

CLIENT	Alon Asphalt Co.	DATE RECEIVED	8/23/23	PROTOCOL	TOX 043 - WDOE
PROJECT	Alon Asphalt Co.	TEST START DATE	8/23/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/26/23	SPECIES	Dendraster excentricus
LAB SAMPLE ID	P230823.01	MATRIX	Liquid	NO. OF ORGANISMS	250

Chronic Toxicity Using Echinoderm Larvae

Concentration (%)	REP	Normal	Abnormal	Date	Tech	Comments/QA Counts
Stocking Density	1	211		8/28/23	MK	Σ = 200, 3
	2	196		8/28/23	MK	
	3	209		8/28/23	MK	
	4	188		8/28/23	MK	
	5	193		8/28/23	MK	
	6	205		8/28/23	MK	
Control	1	178	13	8/28/23	MK	QA: 177N10A Δ 1.6% max
	2	186	11	8/28/23	MK	
	3	202	9	8/28/23	MK	
	4	242	7	8/28/23	MK	
Salt Control	1	199	4	8/28/23	MK	
	2	214	5	8/28/23	MK	
	3	222	5	8/28/23	MK	
	4	207	5	8/28/23	MK	
3.4%	1	214	9	8/28/23	MK	QA 211N13A Δ 1.3% max
	2	222	4	8/28/23	MK	
	3	189	15	8/28/23	MK	
	4	178	2	8/28/23	MK	
11%	1	204	14	8/28/23	MK	
	2	197	6	8/28/23	MK	
	3	209	14	8/28/23	MK	
	4	209	9	8/28/23	MK	
25%	1	199	10	8/28/23	MK	QA 197N9A Δ 0.4% max
	2	199	10	8/28/23	MK	
	3	243	4	8/28/23	MK	
	4	230	8	8/28/23	MK	
50%	1	194	10	8/28/23	MK	
	2	196	6	8/28/23	MK	
	3	222	9	8/28/23	MK	
	4	195	4	8/28/23	MK	
100%	1	211	6	8/28/23	MK	QA 211N4A Δ 0.9% max
	2	199	8	8/28/23	MK	
	3	197	7	8/28/23	MK	
	4	205	7	8/28/23	MK	

CLIENT	Alon Asphalt Co.	DATE RECEIVED	8/23/23	PROTOCOL	TOX 043 - WDOE
PROJECT	Alon Asphalt Co.	TEST START DATE	8/23/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/26/23	SPECIES	<i>Dendraster excentricus</i>
LAB SAMPLE ID	P230823.01	MATRIX	Liquid	NO. OF ORGANISMS	250

Chronic Toxicity Using Echinoderm Larvae

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type
0	0%	0	250.0	250	FSW
	Salt Control	#VALUE!	#VALUE!	250	
	3.4%	8.5	241.5	250	
	11%	27.5	222.5	250	
	25%	62.5	187.5	250	
	50%	125	125.0	250	
	100%	250	0.0	250	

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
8/23/23	7	230823.01	FSW082323.01	J1

**POWER STANDARD CALCULATIONS**

**Echinoderm Development**

Chronic Power Standard Calculation

	average normal/abnormal				
Replicate	1	2	3	4	Mean
CCEC (3.4)	0.9596	0.9823	0.9265	0.9889	0.964325
Control	0.9319	0.9442	0.9573	0.9719	0.951325

Control Mean - CCEC Mean

-0.013

Difference Divided by Control Mean

-0.01366515

Express as %

-1%

≤39% meets the power standard

Pass

## ORGANISM RECEIPT LOG

Date: 8/10/23	Time: 0930	Batch No. DE 081023.01					
Organism: <i>Dendraster excentricus</i>							
Source / Supplier: Field collected							
No. Ordered: 30	No. Received: 30	Source Batch: <u>Collection date, hatch date, etc.:</u> 8/10/23					
Condition of Organisms: Good	Approximate Size or Age: (Days from hatch, life stage, size class, etc.): Adults						
Shipper: counter	B of L (Tracking No.): NA						
Condition of Container: Good	Received By: LG / DM						
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	-	19.6	-	-	-	-	LG / DM
*if >10% contact lab manager							
Notes:							

### MAINTENANCE LOG FOR CULTURES

ORGANISM: De.

LOCATION: Bath 10

Batch Number: De 081023.01      Date Received: 8/10<sup>23</sup>      Initial # of Organisms: 30      10% mortality = 3

Date	Feed AM/PM	Tub No.	D.O.	Temp (°C)	Cond/ Sat	pH	H <sub>2</sub> O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	<sup>1</sup> Cumulative # Mort*	Init.	Comments
8/13	-	1	7.8	16.5	30	7.9	FT	Y	-	0	NL	
8/15	-	1	7.7	16.6	30	7.8	FT	Y	-	0	TW	
8/17	-	1	7.3	16.4	30	7.8	FT	Y	-	0	NL	
8/20	-	1	7.5	15.9	30	7.9	FT	Y	-	0	LG	
8/21	-	1	7.6	15.5	31	7.8	FT	Y	-	0	TW	
8/22	-	1	7.7	15.5	31	7.8	FT	Y	-	0	TW	
8/24	-	1	7.4	17.9	30	7.9	FT	Y	-	0	J1/SR	
8/25	-	1	7.3	17.3	31	7.8	FT	Y	-	0	J1	
8/27/23	-	1	7.4	17.2	31	7.7	FT	Y	-	0	TT	
8/29/23	-	1	7.6	16.2	31	7.9	FT	Y	-	0	J1	
8/30/23	-	1	7.3	16.0	31	7.8	FT	Y	-	0	TT	
8/31/23	-	1	7.4	16.1	31	7.8	FT	Y	-	0	TT	
<del>9/1/23</del>	<del>-</del>	<del>1</del>	<del>16.4</del>	<del>16.3</del>	<del>31</del>	<del>7.8</del>	<del>FT</del>	<del>Y</del>	<del>-</del>	<del>0</del>	<del>J1</del>	
9/1/23	-	1	16.5	16.4	31	7.8	FT	Y	-	0	J1	
9/3/23	-	1	7.7	16.5	31	7.7	FT	Y	-	0	TT	
9/5/23	-	1	7.4	17.5	31	7.9	FT	Y	-	0	J1	
9/6/23	-	1	7.1	16.2	31	7.9	FT	Y	-	0	TW	
09/07/23	-	2	7.9	16.9	31	7.9	FT	Y	0	0	SR	

FT = Flow-through

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the current day's total mortality + previous cumulative culture mortality since acquired in lab

8/8/23 <sup>1</sup> MR - TT 8/22/23

Culture Maintenance Log V1.4

05M J1 8/29/23, 11/9/23

<sup>2</sup> (E) J1 9/1/23 - TT 9/3/23

## **APPENDIX A1.3**

Outfall #1-Grab  
*Atherinops affinis* (Topsmelt)  
96-Hour Acute Test

# CETIS Summary Report

Report Date: 21 Sep-23 17:16 (p 1 of 1)  
 Test Code/ID: P230821.01 A.af / 02-6273-4605

## Fish 96-h Acute Survival Test

EcoAnalysts

<b>Batch ID:</b> 12-9432-6173	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:35	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 25 Aug-23 15:43	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 95h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
18-6187-0693	96h Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	11.3%	1	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
19-4842-8483	96h Proportion Survived	Linear Interpolation (ICPIN)	EC15	>100	---	---	<1	1
			EC20	>100	---	---	<1	
			EC25	>100	---	---	<1	
			EC40	>100	---	---	<1	
			EC50	>100	---	---	<1	

## 96h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	0.00%
0	SC	4	0.9250	0.8454	1.0050	0.9000	1.0000	0.0250	0.0500	5.41%	2.63%
3.4		4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	0.00%
11		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-5.26%
25		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-5.26%
50		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	0.00%
100		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	-2.63%

## 96h Proportion Survived Detail

MD5: 52F369C888E8EDF81DE82B7364F4200D

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	0.8000
0	SC	0.9000	1.0000	0.9000	0.9000
3.4		0.9000	0.9000	1.0000	1.0000
11		1.0000	1.0000	1.0000	1.0000
25		1.0000	1.0000	1.0000	1.0000
50		1.0000	1.0000	1.0000	0.8000
100		1.0000	1.0000	0.9000	1.0000

## 96h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	8/10
0	SC	9/10	10/10	9/10	9/10
3.4		9/10	9/10	10/10	10/10
11		10/10	10/10	10/10	10/10
25		10/10	10/10	10/10	10/10
50		10/10	10/10	10/10	8/10
100		10/10	10/10	9/10	10/10

**CETIS Analytical Report**

Report Date: 21 Sep-23 17:11 (p 1 of 2)  
 Test Code/ID: P230821.01 A.af / 02-6273-4605

**Fish 96-h Acute Survival Test**

EcoAnalysts

<b>Analysis ID:</b> 01-3333-4809	<b>Endpoint:</b> 96h Proportion Survived	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 21 Sep-23 16:38	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 21 Sep-23 16:20	<b>MD5 Hash:</b> 68F12BA7BC724C7A4DB95502F8F631B8	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 12-9432-6173	<b>Test Type:</b> Survival (96h)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:35	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 25 Aug-23 15:43	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 95h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed 96h proportion survived endpoint	10.92%

**Equal Variance t Two-Sample Test**

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	0.5324	1.943	0.1679	CDF	0.3068	Non-Significant Effect

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0042340	0.0042340	1	0.2834	0.6136	Non-Significant Effect
Error	0.0896276	0.0149379	6			
Total	0.0938616		7			

**ANOVA Assumptions Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	1.516	13.75	0.2642	Equal Variances
	Mod Levene Equality of Variance Test	0.1685	13.75	0.6957	Equal Variances
	Variance Ratio F Test	3.499	47.47	0.3310	Equal Variances
Distribution	Anderson-Darling A2 Test	0.6544	3.878	0.0880	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2497	0.3313	0.1612	Normal Distribution
	Shapiro-Wilk W Normality Test	0.8523	0.6451	0.1005	Normal Distribution

**96h Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9500	0.7909	1.0000	1.0000	0.8000	1.0000	0.0500	10.53%	0.00%
0	SC	4	0.9250	0.8454	1.0000	0.9000	0.9000	1.0000	0.0250	5.41%	2.63%

**Angular (Corrected) Transformed Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3360	1.0930	1.5780	1.4120	1.1070	1.4120	0.0762	11.41%	0.00%
0	SC	4	1.2900	1.1600	1.4190	1.2490	1.2490	1.4120	0.0407	6.32%	3.44%

**96h Proportion Survived Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	0.8000
0	SC	0.9000	1.0000	0.9000	0.9000

**Angular (Corrected) Transformed Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.4120	1.4120	1.4120	1.1070
0	SC	1.2490	1.4120	1.2490	1.2490

**CETIS Analytical Report**

Report Date: 21 Sep-23 17:12 (p 2 of 2)  
 Test Code/ID: P230821.01 A.af / 02-6273-4605

**Fish 96-h Acute Survival Test**

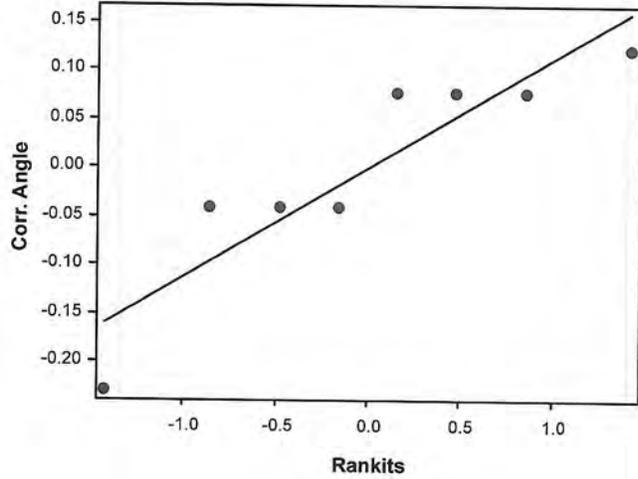
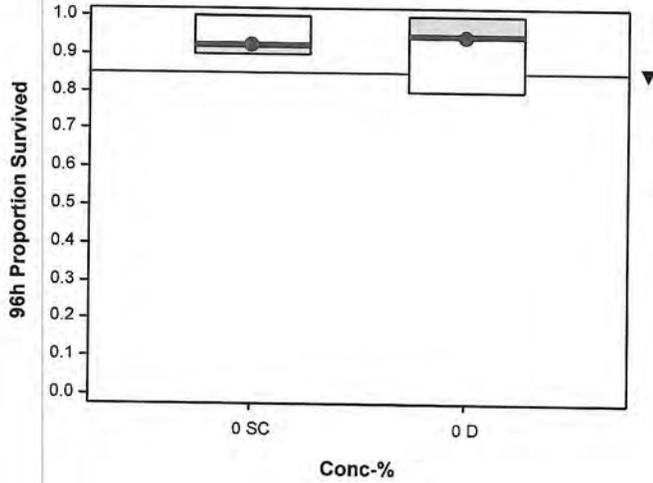
EcoAnalysts

Analysis ID: 01-3333-4809      Endpoint: 96h Proportion Survived      CETIS Version: CETISv2.1.4  
 Analyzed: 21 Sep-23 16:38      Analysis: Parametric-Two Sample      Status Level: 1  
 Edit Date: 21 Sep-23 16:20      MD5 Hash: 68F12BA7BC724C7A4DB95502F8F631B8      Editor ID: 006-677-240-1

**96h Proportion Survived Binomials**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	8/10
0	SC	9/10	10/10	9/10	9/10

**Graphics**



**CETIS Test Data Worksheet**

Report Date: 21 Sep-23 17:19 (p 1 of 1)  
 Test Code/ID: P230821.01 A.af / 02-6273-4605

**Fish 96-h Acute Survival Test**

EcoAnalysts

Start Date: 21 Aug-23 16:35 Species: Atherinops affinis Sample Code: P230821.01  
 End Date: 25 Aug-23 15:43 Protocol: EPA/821/R-02-012 (2002) Sample Source: Alon Asphalt  
 Sample Date: 21 Aug-23 Material: Industrial Effluent Sample Station: Outfall #1 - Grab

Conc-%	Code	Rep	Pos	# Exposed	Survival 24h	Survival 48h	Survival 72h	Survival 96h	Notes
0	D	1	3	10	10	10	10	10	
0	D	2	23	10	10	10	10	10	
0	D	3	10	10	10	10	10	10	
0	D	4	7	10	8	8	8	8	
0	SC	1	5	10	9	9	9	9	
0	SC	2	18	10	10	10	10	10	
0	SC	3	2	10	9	9	9	9	
0	SC	4	15	10	9	9	9	9	
3.4		1	12	10	9	9	9	9	
3.4		2	27	10	9	9	9	9	
3.4		3	4	10	10	10	10	10	
3.4		4	8	10	10	10	10	10	
11		1	22	10	10	10	10	10	
11		2	26	10	10	10	10	10	
11		3	11	10	10	10	10	10	
11		4	16	10	10	10	10	10	
25		1	25	10	10	10	10	10	
25		2	9	10	10	10	10	10	
25		3	13	10	10	10	10	10	
25		4	21	10	10	10	10	10	
50		1	28	10	10	10	10	10	
50		2	19	10	10	10	10	10	
50		3	24	10	10	10	10	10	
50		4	14	10	9	9	9	8	
100		1	6	10	10	10	10	10	
100		2	20	10	10	10	10	10	
100		3	1	10	9	9	9	9	
100		4	17	10	10	10	10	10	

GENERAL

Client	Alon Asphalt Company
Project	Alon Asphalt Company
Project Number	PG1801
Project Manager	M. Seibert
Date Sample Received	8/21/2023
Test type	96-Hour Acute Toxicity with Topsmelt
Matrix	Liquid
Test Acceptability	≥ 90% average survival in control
Test Start Date	08/21/23
Test Species	Atherinops affinis
Organism Batch	ABS081823.01
Organism Acquired	8/18/2023
Organism Acclimation	3
Organism Age	11 days
Test Protocol	TOX001
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 1
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	10
Test Chamber Size	20 oz
Exposure Volume	250 mL
Feeding Information	0.1mL Artemia 1x day
Test Dissolved Oxygen	> 4.0
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	19	21
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 1635 LG, mg  
 TEST END TIME/INIT: 1543 SR

CLIENT SAMPLE ID	LAB ID
Outfall #1- Grab	P230821.01

Concentrations	
1	Control
2	Salt Control
3	3.4%
4	11%
5	25%
6	50%
7	100%
8	.
9	.

Food Batch ID
281729

CSMM Batch #
A2017620

Copy and Past VALUES from

Treatment	Rep	Chamber
Control	1	17
Control	2	26
Control	3	7
Control	4	28
Salt Control	1	15
Salt Control	2	25
Salt Control	3	5
Salt Control	4	3
3.4%	1	8
3.4%	2	21
3.4%	3	1
3.4%	4	19
11%	1	10
11%	2	4
11%	3	14
11%	4	18
25%	1	13
25%	2	20
25%	3	2
25%	4	16
50%	1	11
50%	2	24
50%	3	27
50%	4	23
100%	1	22
100%	2	6
100%	3	9
100%	4	12

V.4 CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX001
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/25/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with Topsmelt

	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH	
<b>Concentration (%)</b>	> 4.0	19 - 21	28 - 32	6 - 9	
<b>Day 0</b>					
Stock	Control	7.6	20.1	31	8.0
Date 8/21/23	Salt Control	7.5 7.3	19.0	36 30	8.3
Time 1515	3.4%	7.7	20.0	31 30	8.0
Tech TW	11%	7.7	19.3	31	8.0
Meter # 7	25%	7.8	19.4	31	8.0
Feed: LG	50%	8.1	19.7	30	8.0
	100%	8.7	19.9	30	7.9
<b>Day 1</b>					
Rep 1	Control	6.4	20.2	31	7.9
Date 8/22/23	Salt Control	6.7	20.3	30	8.1
Time 1028	3.4%	6.6	20.2	31	7.9
Tech J1	11%	6.4	20.1	31	8.0
Meter # 9	25%	7.0	19.9	31	8.0
	50%	6.9	19.9	31	8.0
	100%	6.9	20.0	30	8.1
<b>Day 2</b>					
Rep 2	Control	6.3	20.1	31	7.8
Date 8/23/23	Salt Control	6.4	20.4	30	8.0
Time 0955	3.4%	7.0	20.1	31	7.9
Tech J1	11%	6.4	20.1	31	7.9
Meter # 9	25%	6.8	19.9	31	7.9
	50%	6.2	20.5	31	8.0
	100%	6.4	20.2	30	8.2
<b>Day 2</b>					
Renewal Stock	Control	7.5	19.6	31	8.1
Date 8/23/23	Salt Control	7.4 7.5	19.5 20.1	31 30	8.1 8.3
Time 1005	3.4%	7.6	19.3	31	8.1
Tech J1	11%	7.5	19.4	31	8.3 8.1
Meter # 9	25%	7.8	19.4	31	8.1
	50%	8.0	19.4	30	8.1
	100%	8.3	19.9	30	7.6

① I.E, TW 8/21/23, J1 8/23/23

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX001
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/25/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with Topsmelt

	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
<b>Concentration (%)</b>	> 4.0	19 - 21	28 - 32	6 - 9
<b>Day 3</b>				
Rep 3				
Date 8/24/23				
Time 1137				
Tech J1				
Meter # 9				
Control	6.5	20.4	31	7.8
Salt Control	6.7	20.0	31	8.1
3.4%	6.5	20.4	31	7.9
11%	6.5	20.4	31	7.9
25%	6.6	20.4	31	8.0
50%	6.8	20.4	31	8.1
100%	7.0	20.4	30	8.2
<b>Day 4</b>				
Rep 4				
Date 8/25/23				
Time 1133				
Tech J1				
Meter # 9				
Control	6.5	20.4	31	7.7
Salt Control	6.6	20.1	31	8.0
3.4%	6.6	20.1	31	7.8
11%	6.3	20.2	31	7.9
25%	6.4	20.1	31	7.9
50%	6.2	20.4	31	8.0
100%	6.5	20.2	30	8.2

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX001
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/25/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

Abbreviation Key:

NB = No Body

FB = Found Body

ST = Stranded

96-Hour Acute Toxicity with Topsmelt

Concentration (%)	Rep	Day 1		Day 2		Day 3		Day 4	
		Date	8/22	Date	8/23	Date	8/24	Date	08/25/23
		Time	1041	Time	1050	Time	1137	Time	1543
		Tech	J1	Tech	J1	Tech	J1	Tech	SR
Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	8	2	8	0	8	0	8	0
Salt Control	1	9	1	9	0	9	0	9	0
	2	10	0	10	0	10	0	10	0
	3	9	1	9	0	9	0	9	0
	4	9	1	9	0	9	0	9	0
3.4%	1	9	1	9	0	9	0	9	0
	2	9	1	9	0	9	0	9	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
11%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
25%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
50%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
100%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	9	1	9	0	9	0	9	0
	4	10	0	10	0	10	0	10	0
Feed (Init.)	AM	TW		TW		UG		NL	
0.1mL Artemia 1x day	PM								

① IC J1 8/22/23

V.4

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX001
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/25/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	10

96-Hour Acute Toxicity with Topsmelt

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	1000.0	1000	FSW	
	Salt Control	#VALUE!	#VALUE!	1000		
	3.4%	34	966.0	1000		
	11%	110	890.0	1000		
	25%	250	750.0	1000		
	50%	500	500.0	1000		
	100%	1000	0.0	1000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
2	0%	0	800.0	800
	Salt Control	#VALUE!	#VALUE!	800
	3.4%	27.2	772.8	800
	11%	88	712.0	800
	25%	200	600.0	800
	50%	400	400.0	800
	100%	800	0.0	800

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
8/21/23	7	P230821.01	FSW081323.01	LG
8/23/23	7	P230821.01	FSW081323.0	SI/NL

① IE: Actual: FSW082023.01 - NL 8/23/23

## POWER STANDARD CALCULATIONS

*Atherinops affinis* Acute Survival

Acute Power Standard Calculation

Replicate	Number Surviving				Mean
	1	2	3	4	
ACEC (11)	10	10	10	10	10
Control	10	10	10	8	9.5

Control Mean - ACEC Mean

-0.5

Difference Divided by Control Mean

-0.05263158

Express as %

-5%

≤29% meets the power standard

Pass

## ORGANISM RECEIPT LOG

<b>Date:</b> 8/10/23	<b>Time:</b> 1230	<b>Batch No.</b> ABS081823.01					
<b>Organism:</b> <p style="text-align: center; font-size: 1.2em;">Atherinops affinis</p>							
<b>Source / Supplier:</b> <p style="text-align: center; font-size: 1.2em;">Aquatic Biosystems</p>							
<b>No. Ordered:</b> <del>610</del> <sup>①</sup> 560	<b>No. Received:</b> 610	<b>Source Batch:</b> Collection date, hatch date, etc.): Hatch: 8/10/23					
<b>Condition of Organisms:</b> <p style="text-align: center; font-size: 1.2em;">Good</p>		<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): <p style="text-align: center; font-size: 1.2em;">8 days</p>					
<b>Shipper:</b> <p style="text-align: center; font-size: 1.2em;">FedEx</p>		<b>B of L (Tracking No.)</b> 137496024119					
<b>Condition of Container:</b> <p style="text-align: center; font-size: 1.2em;">Good</p>		<b>Received By:</b> <p style="text-align: center; font-size: 1.2em;">M. Seibert</p>					
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	10.7	20.9	29 ppt	7.2	20	-	NS
2	10.2	20.9	29 ppt	7.1	20	Total: 40 7.5%	NS
*if >10% contact lab manager							
<b>Notes:</b>							

① WC-MS 8/10

1300 Blue Spruce Drive, Suite C  
Fort Collins, Colorado 80524



Toll Free: 800/331-5916  
Tel: 970/484-5091 Fax: 970/484-2514

### ORGANISM HISTORY

DATE: 8/17/2023

SPECIES: Atherinops affinis

AGE: 7 day

LIFE STAGE: Larvae

HATCH DATE: 8/10/2023

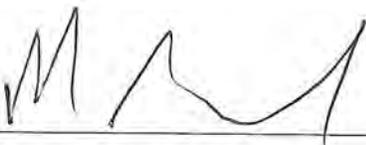
BEGAN FEEDING: Immediately

FOOD: Artemia sp.

### Water Chemistry Record:

	Mean	Range
TEMPERATURE:	<u>19°C</u>	<u>--</u>
SALINITY/CONDUCTIVITY:	<u>30 ppt</u>	<u>--</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>140 mg/l</u>	<u>--</u>
pH:	<u>7.60</u>	<u>--</u>

### Comments:

  
\_\_\_\_\_  
*Facility Supervisor*

## MAINTENANCE LOG FOR CULTURES

ORGANISM: Topples  
 LOCATION: Bath 1

Batch Number: ABS081823.01    Date Received: 8/18/23    Initial # of Organisms: 610    10% mortality = 61

Date	Feed AM/PM	Tub No.	D.O.	Temp (°C)	Cond/ (Sal)	pH	H <sub>2</sub> O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	<sup>1</sup> Cumulative # Mort*	Init.	Comments
8/19	✓ ✓	A	6.8	19.5	30	7.8	Y	Y	0	—	NL	
8/19	✓ ✓	B	6.8	19.5	30	7.8	Y	Y	4	—	NL	
8/19	✓ ✓	C	6.9	19.6	30	7.8	Y	Y	0	4	NL	
8/20	✓ ✓	A	6.9	19.5	30	7.7	Y	Y	10	—	LG	
8/20	✓ ✓	B	7.0	19.5	30	7.8	Y	Y	0	—	LG	
8/20	✓ ✓	C	6.8	19.7	30	7.7	Y	Y	0	14	LG	
8/21	✓ ✓	A	7.0	19.0	31	7.8	N	Y	0	—	TW	
8/21	✓ ✓	B	6.9	19.0	31	7.9	N	Y	0	—	TW	
8/21	✓ ✓	C	7.0	19.2	31	7.8	N	Y	0	14	TW	
8/22	✓	A	7.3	19.1	31	7.9	Y	Y	102	116	TW	condesed A,B,C to A
<del>Finished Culture</del>												

FT = Flow-through

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the current day's total mortality + previous cumulative culture mortality since acquired in lab

8/8/23

Culture Maintenance Log V1.4

## **APPENDIX A1.4**

Outfall #1-Grab  
*Atherinops affinis* (Topsmelt)  
7-Day Chronic Test

# CETIS Summary Report

Report Date: 21 Sep-23 20:26 (p 1 of 3)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

<b>Batch ID:</b> 11-3393-6778	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:36	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 28 Aug-23 16:49	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 7d 0h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
17-7174-1849	7d Proportion Survived	Steel Many-One Rank Sum Test	100	>100	---	11.9%	1	1
15-2744-5988	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test	100	>100	---	24.4%	1	1
00-1109-8992	Mean Dry Weight-mg	Dunnett Multiple Comparison Test	100	>100	---	16.9%	1	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
20-4604-8159	7d Proportion Survived	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
05-1575-9127	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓ IC15	>100	---	---	<1	1
			✓ IC20	>100	---	---	<1	
			✓ IC25	>100	---	---	<1	
			✓ IC40	>100	---	---	<1	
			✓ IC50	>100	---	---	<1	
01-5263-9741	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	✓ IC15	>100	---	---	<1	1
			✓ IC20	>100	---	---	<1	
			✓ IC25	>100	---	---	<1	
			✓ IC40	>100	---	---	<1	
			✓ IC50	>100	---	---	<1	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits			Decision
				Lower	Upper	Overlap	
17-7174-1849	7d Proportion Survived	Control Resp	0.92	0.8	<<	Yes	Passes Criteria
20-4604-8159	7d Proportion Survived	Control Resp	0.92	0.8	<<	Yes	Passes Criteria
05-1575-9127	Mean Dry Biomass-mg	Control Resp	0.854	0.85	<<	Yes	Passes Criteria
15-2744-5988	Mean Dry Biomass-mg	Control Resp	0.854	0.85	<<	Yes	Passes Criteria
17-7174-1849	7d Proportion Survived	PMSD	0.1192	<<	0.25	No	Passes Criteria
15-2744-5988	Mean Dry Biomass-mg	PMSD	0.2438	<<	0.5	No	Passes Criteria

**CETIS Summary Report**

Report Date: 21 Sep-23 20:26 (p 2 of 3)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

**7d Proportion Survived Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.9200	0.7840	1.0560	0.8000	1.0000	0.0490	0.1095	11.91%	0.00%
0	SC	5	0.8800	0.7440	1.0160	0.8000	1.0000	0.0490	0.1095	12.45%	4.35%
3.4		5	0.9600	0.8489	1.0710	0.8000	1.0000	0.0400	0.0894	9.32%	-4.35%
11		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-8.70%
25		5	0.9200	0.7840	1.0560	0.8000	1.0000	0.0490	0.1095	11.91%	0.00%
50		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-8.70%
100		5	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-8.70%

**Mean Dry Biomass-mg Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.854	0.6593	1.049	0.714	1.08	0.07013	0.1568	18.36%	0.00%
0	SC	5	1.019	0.8402	1.197	0.8	1.136	0.06432	0.1438	14.12%	-19.30%
3.4		5	1.014	0.7651	1.263	0.696	1.192	0.08964	0.2004	19.77%	-18.74%
11		5	0.9716	0.8813	1.062	0.876	1.06	0.03254	0.07275	7.49%	-13.77%
25		5	0.8092	0.5804	1.038	0.618	1.036	0.08241	0.1843	22.77%	5.25%
50		5	0.898	0.824	0.972	0.84	0.972	0.02667	0.05963	6.64%	-5.15%
100		5	0.9988	0.8812	1.116	0.86	1.102	0.04237	0.09475	9.49%	-16.96%

**Mean Dry Weight-mg Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.9302	0.7631	1.097	0.714	1.08	0.06019	0.1346	14.47%	0.00%
0	SC	5	1.17	0.9101	1.429	0.948	1.42	0.09346	0.209	17.87%	-25.74%
3.4		5	1.049	0.8796	1.218	0.87	1.192	0.06094	0.1363	12.99%	-12.75%
11		5	0.9716	0.8813	1.062	0.876	1.06	0.03254	0.07275	7.49%	-4.45%
25		5	0.8714	0.7348	1.008	0.7725	1.036	0.0492	0.11	12.62%	6.32%
50		5	0.898	0.824	0.972	0.84	0.972	0.02667	0.05963	6.64%	3.46%
100		5	0.9988	0.8812	1.116	0.86	1.102	0.04237	0.09475	9.49%	-7.37%

**CETIS Summary Report**

Report Date: 21 Sep-23 20:26 (p 3 of 3)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

**7d Proportion Survived Detail**

MD5: 8151C14A92D3ACCFCD22685E83D74084

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	0.8000	0.8000
0	SC	1.0000	0.8000	0.8000	1.0000	0.8000
3.4		0.8000	1.0000	1.0000	1.0000	1.0000
11		1.0000	1.0000	1.0000	1.0000	1.0000
25		1.0000	0.8000	1.0000	1.0000	0.8000
50		1.0000	1.0000	1.0000	1.0000	1.0000
100		1.0000	1.0000	1.0000	1.0000	1.0000

**Mean Dry Biomass-mg Detail**

MD5: B269D2AA552944FE3A2D83D4E4AE3434

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.952	0.714	1.08	0.786	0.738
0	SC	1.13	1.08	1.136	0.948	0.8
3.4		0.696	0.998	1.192	1.18	1.004
11		0.876	1.014	1.06	0.924	0.984
25		1.036	0.626	0.842	0.924	0.618
50		0.84	0.866	0.86	0.952	0.972
100		1.046	0.95	0.86	1.102	1.036

**Mean Dry Weight-mg Detail**

MD5: 6F07EC569B0A05E89B2A005E3D6BF503

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.952	0.714	1.08	0.9825	0.9225
0	SC	1.13	1.35	1.42	0.948	1
3.4		0.87	0.998	1.192	1.18	1.004
11		0.876	1.014	1.06	0.924	0.984
25		1.036	0.7825	0.842	0.924	0.7725
50		0.84	0.866	0.86	0.952	0.972
100		1.046	0.95	0.86	1.102	1.036

**7d Proportion Survived Binomials**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	5/5	5/5	5/5	4/5	4/5
0	SC	5/5	4/5	4/5	5/5	4/5
3.4		4/5	5/5	5/5	5/5	5/5
11		5/5	5/5	5/5	5/5	5/5
25		5/5	4/5	5/5	5/5	4/5
50		5/5	5/5	5/5	5/5	5/5
100		5/5	5/5	5/5	5/5	5/5

# CETIS Analytical Report

Report Date: 21 Sep-23 20:31 (p 1 of 6)  
 Test Code/ID: P230821.01A.afc / 19-3721-1715

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

<b>Analysis ID:</b> 11-5185-5649	<b>Endpoint:</b> 7d Proportion Survived	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 21 Sep-23 18:27	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 21 Sep-23 18:08	<b>MD5 Hash:</b> 7DDDDDEDA7ACDCF7C103614BC5A936	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 11-3393-6778	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:36	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 28 Aug-23 16:49	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 7d 0h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed 7d proportion survived endpoint	13.97%

### Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	8	0.5774	1.86	0.1534	CDF	0.2898	Non-Significant Effect

### Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.88	0.8	<<	Yes	Passes Criteria
Control Resp	0.92	0.8	<<	Yes	Passes Criteria
PMSD	0.1397	<<	0.25	No	Passes Criteria

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0056708	0.0056708	1	0.3333	0.5796	Non-Significant Effect
Error	0.136099	0.0170124	8			
Total	0.14177		9			

### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	0	11.26	1.0000	Equal Variances
	Mod Levene Equality of Variance Test	0	13.75	1.0000	Equal Variances
	Variance Ratio F Test	1	23.15	1.0000	Equal Variances
Distribution	Anderson-Darling A2 Test	1.063	3.878	0.0087	Non-Normal Distribution
	D'Agostino Skewness Test	0	2.576	1.0000	Normal Distribution
	Kolmogorov-Smirnov D Test	0.2807	0.3025	0.0247	Normal Distribution
	Shapiro-Wilk W Normality Test	0.7993	0.7411	0.0142	Normal Distribution

### 7d Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9200	0.7840	1.0000	1.0000	0.8000	1.0000	0.0490	11.91%	0.00%
0	SC	5	0.8800	0.7440	1.0000	0.8000	0.8000	1.0000	0.0490	12.45%	4.35%

### Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.2500	1.0880	1.4120	1.3450	1.1070	1.3450	0.0583	10.43%	0.00%
0	SC	5	1.2020	1.0400	1.3640	1.1070	1.1070	1.3450	0.0583	10.85%	3.81%

### 7d Proportion Survived Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	1.0000	0.8000	0.8000
0	SC	1.0000	0.8000	0.8000	1.0000	0.8000



**CETIS Analytical Report**

Report Date: 21 Sep-23 20:32 (p 3 of 6)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

<b>Analysis ID:</b> 18-8790-9136	<b>Endpoint:</b> Mean Dry Biomass-mg	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 21 Sep-23 18:27	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 21 Sep-23 18:08	<b>MD5 Hash:</b> 88DA5F4E06EFC1E774E270F46CE7B98D	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 11-3393-6778	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:36	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 28 Aug-23 16:49	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 7d 0h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	Salt Control passed mean dry biomass-mg endpoint	20.72%

**Equal Variance t Two-Sample Test**

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	8	-1.732	1.86	0.1769	CDF	0.9392	Non-Significant Effect

**Test Acceptability Criteria**

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	1.019	0.85	<<	Yes	Passes Criteria
Control Resp	0.854	0.85	<<	Yes	Passes Criteria
PMSD	0.2072	<<	0.5	No	Passes Criteria

**ANOVA Table**

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0678975	0.0678975	1	2.999	0.1215	Non-Significant Effect
Error	0.181093	0.0226366	8			
Total	0.24899		9			

**ANOVA Assumptions Tests**

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	0.1262	11.26	0.7316	Equal Variances
	Mod Levene Equality of Variance Test	0.04018	13.75	0.8478	Equal Variances
	Variance Ratio F Test	1.189	23.15	0.8709	Equal Variances
Distribution	Anderson-Darling A2 Test	0.3654	3.878	0.4404	Normal Distribution
	D'Agostino Skewness Test	0.01833	2.576	0.9854	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1842	0.3025	0.4901	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9483	0.7411	0.6486	Normal Distribution

**Mean Dry Biomass-mg Summary**

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.854	0.6593	1.049	0.786	0.714	1.08	0.07013	18.36%	0.00%
0	SC	5	1.019	0.8402	1.197	1.08	0.8	1.136	0.06432	14.12%	-19.30%

**Mean Dry Biomass-mg Detail**

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.952	0.714	1.08	0.786	0.738
0	SC	1.13	1.08	1.136	0.948	0.8



# CETIS Analytical Report

Report Date: 21 Sep-23 20:32 (p 5 of 6)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

<b>Analysis ID:</b> 13-1749-5507	<b>Endpoint:</b> Mean Dry Weight-mg	<b>CETIS Version:</b> CETISv2.1.4
<b>Analyzed:</b> 21 Sep-23 18:27	<b>Analysis:</b> Parametric-Two Sample	<b>Status Level:</b> 1
<b>Edit Date:</b> 21 Sep-23 18:08	<b>MD5 Hash:</b> 382419714534F86FC8DC68DB99935A36	<b>Editor ID:</b> 006-677-240-1
<b>Batch ID:</b> 11-3393-6778	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b>
<b>Start Date:</b> 21 Aug-23 16:36	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 28 Aug-23 16:49	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Crystal Sea Marine Mix
<b>Test Length:</b> 7d 0h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 11d
<b>Sample ID:</b> 12-7990-1654	<b>Code:</b> P230821.01	<b>Project:</b> Alon Asphalt Company
<b>Sample Date:</b> 21 Aug-23	<b>Material:</b> Industrial Effluent	<b>Source:</b> Alon Asphalt (WA0003239)
<b>Receipt Date:</b> 21 Aug-23 13:05	<b>CAS (PC):</b>	<b>Station:</b> Outfall #1 - Grab
<b>Sample Age:</b> 17h (2.1 °C)	<b>Client:</b> Alon Asphalt	

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	Salt Control passed mean dry weight-mg endpoint	22.22%

### Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	8	-2.154	1.86	0.2067	CDF	0.9683	Non-Significant Effect

### ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.143281	0.143281	1	4.638	0.0634	Non-Significant Effect
Error	0.247135	0.0308919	8			
Total	0.390416		9			

### ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	2.339	11.26	0.1647	Equal Variances
	Mod Levene Equality of Variance Test	2.731	13.75	0.1495	Equal Variances
	Variance Ratio F Test	2.411	23.15	0.4149	Equal Variances
Distribution	Anderson-Darling A2 Test	0.282	3.878	0.6671	Normal Distribution
	D'Agostino Skewness Test	0.03882	2.576	0.9690	Normal Distribution
	Kolmogorov-Smirnov D Test	0.147	0.3025	0.9925	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9408	0.7411	0.5622	Normal Distribution

### Mean Dry Weight-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9302	0.7631	1.097	0.952	0.714	1.08	0.06019	14.47%	0.00%
0	SC	5	1.17	0.9101	1.429	1.13	0.948	1.42	0.09346	17.87%	-25.74%

### Mean Dry Weight-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.952	0.714	1.08	0.9825	0.9225
0	SC	1.13	1.35	1.42	0.948	1



CETIS Test Data Worksheet

Report Date: 21 Sep-23 20:35 (p 1 of 2)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

EcoAnalysts

Topsmelt 7-d Survival and Growth Test

Start Date: 21 Aug-23 16:36 Species: Atherinops affinis Sample Code: P230821.01  
 End Date: 28 Aug-23 16:49 Protocol: EPA/600/R-95/136 (1995) Sample Source: Alon Asphalt  
 Sample Date: 21 Aug-23 Material: Industrial Effluent Sample Station: Outfall #1 - Grab

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Weight-mg Total	Weight-mg Tare	Pan Count	Notes
0	D	1	7	5	5	5	5	5	5	5	5	30.54	25.78	5	
0	D	2	26	5	5	5	5	5	5	5	5	34.37	30.8	5	
0	D	3	18	5	5	5	5	5	5	5	5	32.48	27.08	5	
0	D	4	20	5	4	4	4	4	4	4	4	36.24	32.31	4	
0	D	5	17	5	4	4	4	4	4	4	4	31.48	27.79	4	
0	SC	1	25	5	5	5	5	5	5	5	5	35.51	29.86	5	
0	SC	2	8	5	4	4	4	4	4	4	4	38.01	32.61	4	
0	SC	3	35	5	5	5	5	5	5	4	4	27.12	21.44	4	
0	SC	4	19	5	5	5	5	5	5	5	5	25.41	20.67	5	
0	SC	5	14	5	4	4	4	4	4	4	4	24.41	20.41	4	
3.4		1	12	5	4	4	4	4	4	4	4	29.02	25.54	4	
3.4		2	24	5	5	5	5	5	5	5	5	28.56	23.57	5	
3.4		3	9	5	5	5	5	5	5	5	5	28.71	22.75	5	
3.4		4	11	5	5	5	5	5	5	5	5	28.77	22.87	5	
3.4		5	23	5	5	5	5	5	5	5	5	28.66	23.64	5	
11		1	13	5	5	5	5	5	5	5	5	26.32	21.94	5	
11		2	15	5	5	5	5	5	5	5	5	30.89	25.82	5	
11		3	22	5	5	5	5	5	5	5	5	28.13	22.83	5	
11		4	10	5	5	5	5	5	5	5	5	28.72	24.1	5	
11		5	32	5	5	5	5	5	5	5	5	27.38	22.46	5	
25		1	30	5	5	5	5	5	5	5	5	27.84	22.66	5	
25		2	27	5	5	5	5	5	5	4	4	26.13	23	4	
25		3	1	5	5	5	5	5	5	5	5	27.17	22.96	5	
25		4	33	5	5	5	5	5	5	5	5	31.83	27.21	5	
25		5	4	5	4	4	4	4	4	4	4	30.96	27.87	4	
50		1	29	5	5	5	5	5	5	5	5	27.4	23.2	5	
50		2	34	5	5	5	5	5	5	5	5	29.59	25.26	5	
50		3	3	5	5	5	5	5	5	5	5	36.31	32.01	5	

CETIS Test Data Worksheet

Report Date: 21 Sep-23 20:35 (p 2 of 2)  
 Test Code/ID: P230821.01A.afC / 19-3721-1715

Conc-%	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Notes
50		4	2	5	5	5	5	5	5	5	5	26.77	22.01	5	
50		5	28	5	5	5	5	5	5	5	5	21.99	17.13	5	
100		1	6	5	5	5	5	5	5	5	5	23.38	18.15	5	
100		2	31	5	5	5	5	5	5	5	5	22.84	18.09	5	
100		3	5	5	5	5	5	5	5	5	5	21.61	17.31	5	
100		4	16	5	5	5	5	5	5	5	5	22.96	17.45	5	
100		5	21	5	5	5	5	5	5	5	5	24.45	19.27	5	

GENERAL

Client	Alon Asphalt Company
Project	Alon Asphalt Company
Project Number	PG1801
Project Manager	M. Seibert
Date Sample Received	8/21/2023
Test type	7 Day Chronic Toxicity with Topsmelt
Matrix	Liquid
Test Acceptability	≥ 80% average survival in control Average dry weight is > 0.85 mg per surviving fish
Test Start Date	08/21/23
Test Species	Atherinops affinis
Organism Batch	ABS081823.01
Organism Acquired	8/18/2023
Organism Acclimation	3
Organism Age	11 days
Test Protocol	TOX 002
Regional Protocol	WDOE WQ-R-95-80
Test Location	Bath 9
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	5
Test Chamber Size	20 oz.
Exposure Volume	250 mL
Feeding Information	250 nauplii/chamber am 500 nauplii/chamber pm
Test Dissolved Oxygen	> 4.0
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	19	21
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 1636 MS, LG  
TEST END TIME/INIT: 1649 JI

CLIENT SAMPLE ID	LAB ID
Outfall #1- Grab	P230821.01
Outfall #1- Grab	P230823.01
Outfall #1- Grab	P230825.01

Concentrations	
1	Control
2	Salt Control
3	3.4%
4	11%
5	25%
6	50%
7	100%
8	.
9	.

Food Batch ID
281729

CSMM Batch #
A2017620

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	34
Control	2	7
Control	3	17
Control	4	21
Control	5	2
Salt Control	1	12
Salt Control	2	20
Salt Control	3	13
Salt Control	4	26
Salt Control	5	10
3.4%	1	30
3.4%	2	15
3.4%	3	4
3.4%	4	22
3.4%	5	35
11%	1	29
11%	2	32
11%	3	3
11%	4	16
11%	5	8
25%	1	19
25%	2	27
25%	3	28
25%	4	24
25%	5	23
50%	1	33
50%	2	1
50%	3	14
50%	4	6
50%	5	9
100%	1	18
100%	2	31
100%	3	25
100%	4	5
100%	5	11

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

7 Day Chronic Toxicity with Topsmelt

	Concentration (%)	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
		> 4.0	19 - 21	28 - 32	6 - 9
<b>Day 0</b>	Control	7.6	19.1	31	8.0
Stock	Salt Control	7.3	19.0	30	8.3
Date 8/21/23	3.4%	7.5	20.9	31	7.9
Time 1605	11%	7.7	19.4	31	8.0
Tech TW	25%	7.9	19.5	31	8.0
Meter # 7	50%	7.9	20.4	30	7.9
Feed LG	100%	8.5	19.9	30	8.0
<b>Day 1</b>	Control	7.5	19.0	31	7.8
Rep 1	Salt Control	7.5	19.2	30	8.1
Date 8/22/23	3.4%	7.6	18.9	31	8.0
Time 1335	11%	7.7	18.5	31	8.0
Tech TW	25%	7.8	19.8	31	8.1
Meter # 7	50%	8.2	20.7	30	8.0
	100%	8.8	20.2	30	8.0
<b>Day 1</b>	Control	7.7	19.9	31	7.8
Renewal Stock	Salt Control	7.6	19.4	30	7.8
Date 8/22/23	3.4%	7.8	20.2	31	8.0
Time 1340	11%	7.9	20.2	31	7.9
Tech TW	25%	7.9	20.1	30	8.0
Meter # 7	50%	8.3	19.9	30	7.8
	100%	8.2	18.9	30	8.1
<b>Day 2</b>	Control	6.7	19.9	31	7.6
Rep 2	Salt Control	6.4	19.9	31	8.0
Date 8/23/23	3.4%	6.4	20.1	31	7.9
Time 1332	11%	6.5	20.0	31	7.9
Tech LG	25%	6.7	19.9	31	8.0
Meter # 7	50%	6.7	19.9	31	8.1
	100%	6.5	20.0	30	8.1
<b>Day 2</b>	Control	7.6	19.2	31	8.1
Renewal Stock	Salt Control	7.6	19.3	30	8.3
Date 8/23/23	3.4%	7.6	19.0	31	8.1
Time 1346	11%	7.7	18.9	31	8.1
Tech LG	25%	7.9	18.6	31	8.1
Meter # 7	50%	8.1	19.0	31	8.1
	100%	8.7	19.3	30	8.1

V.4 CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

7 Day Chronic Toxicity with Topsmelt

	Concentration (%)	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
		> 4.0	19 - 21	28 - 32	6 - 9
Day 3	Control	6.4	20.2	31.0	7.8
Rep 3	Salt Control	6.4	20.2	30	8.1
Date 8/24/23	3.4%	6.4	20.2	31	7.9
Time 1031	11%	6.5	20.3	31	7.9
Tech Π	25%	6.5	20.1	30	7.9
Meter # 9	50%	6.7	20.1	30	8.1
	100%	6.8	20.3	29	8.2
Day 3	Control	7.4	19.5	31	8.1
Renewal Stock	Salt Control	7.4	19.8	30	8.3
Date 8/24/23	3.4%	7.3	19.5	31	8.1
Time 1053	11%	7.5	21.3	31	8.1
Tech Π	25%	7.4	20.4	30	8.1
Meter # 9	50%	8.3	21.1	31	8.2
	100%	8.2	20.9	30	8.2
Day 4	Control	6.4	20.2	31	7.9
Rep 4	Salt Control	6.8	20.1	30	8.1
Date 8/25/23	3.4%	5.7	20.1	31	7.8
Time 0929	11%	6.0	20.1	31	7.9
Tech Π	25%	6.2	20.1 <sup>(2)</sup> 20.2	31	8.0
Meter # 9	50%	6.0	20.3	31	8.1
	100%	6.0	20.3	30	8.2
Day 4	Control	7.2	19.6	31	8.1
Renewal Stock	Salt Control	7.3	19.9	30	8.3
Date 8/25/23	3.4%	7.3	19.9	30	8.1
Time 0959	11%	7.2	20.2	31	8.1
Tech Π	25%	7.4 <sup>(2)</sup> 7.7	20.2	31	8.1
Meter # 9	50%	8.0	19.9	30	8.2
	100%	8.2 <sup>(2)</sup> 8.6	19.6	31	8.2
Day 5	Control	6.5	19.8	31	7.7
Rep 5	Salt Control	6.5	19.8	30	8.0
Date 8/26/23	3.4%	6.9	19.9	31	7.9
Time 0843	11%	6.5	19.7	31	7.9
Tech Π	25%	7.2	19.4	31	8.0
Meter # 7	50%	6.6	19.4	31	8.1
	100%	6.3	19.5	31	8.2

① S.M. - Π 8/24/23  
 ② I.E. - Π 8/25/23, Π 8/25/23

V.4 CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

7 Day Chronic Toxicity with Topsmelt

	Concentration (%)	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
		> 4.0	19 - 21	28 - 32	6 - 9
<b>Day 5</b>	Control	7.3	19.6	31	8.0
Renewal Stock	Salt Control	7.5	20.0	30	8.2
Date 8/26/23	3.4%	7.5	19.5	31	8.1
Time 1038	11%	7.6	19.7	31	8.1
Tech Π	25%	7.7	19.7	30	8.0
Meter # 9	50%	7.9	20.8	29	8.0
	100%	8.1	20.7	28	8.0
<b>Day 6</b>	Control	6.3	20.0	30	7.6
Rep 1	Salt Control	6.9	20.0	31	8.0
Date 8/27/23	3.4%	6.5	19.9	31	7.9
Time 1250	11%	7.1	19.7	31	7.9
Tech Π	25%	6.6.6	19.9	30	7.9
Meter # 7	50%	6.9	19.8	30	8.0
	100%	7.0	19.7	29	8.0
<b>Day 6</b>	Control	7.6	19.3	31	8.0
Renewal Stock	Salt Control	7.6	19.7	30	8.2
Date 8/27/23	3.4%	7.6	19.5	31	8.0
Time 1305	11%	7.6	19.7	31	8.0
Tech Π	25%	7.7	19.5	31	8.0
Meter # 7	50%	7.7	19.7	30	8.0
	100%	8.07.8	19.6	29	8.0
<b>Day 7</b>	Control	6.2	20.2	31	7.8
Rep 2	Salt Control	6.4	20.2	31	8.1
Date 8/28/23	3.4%	6.5	20.3	31	7.8
Time 1000	11%	6.5	20.1	31	7.9
Tech J1	25%	6.8	20.1	31	8.0
Meter # 8	50%	6.9	20.0	31	8.0
	100%	6.8	20.1	29	8.1

① 1E - Π 8/27/23

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	Atherinops affinis
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

Abbreviation Key:  
 NB = No Body  
 FB = Found Body  
 ST = Stranded

7 Day Chronic Toxicity with Topsmelt

Concentration (%)	REP	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		Date	Time												
		Tech		Tech		Tech		Tech		Tech		Tech		Tech	
Control	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
Salt Control	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
3.4%	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
11%	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
25%	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
50%	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
100%	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
Feed (Init.)	AM	TW		TW		LG		NL		TT		TT		NONE	
250 nauplii/chamber am 500 nauplii/chamber pm	PM	TW		JI		JI		TT		TT		TT		NONE	

① IE - TT 8/26/23

v.4

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

**7 Day Chronic Toxicity with Topsmelt**

Concentration (%)	REP	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count
Control	1	1	25.78	30.54	5
	2	2	30.80	34.37	5
	3	3	27.08	32.48	5
	4	4	32.31	36.24	4
	5	5	27.79	31.48	4
Salt Control	1	6	29.86	35.51	5
	2	7	32.41	38.01	4
	3	8	21.44	27.12	4
	4	9	20.67	25.41	5
	5	10	20.41	24.41	4
3.4%	1	11	25.54	29.02	4
	2	12	23.57	28.56	5
	3	13	22.75	28.71	5
	4	14	22.87	28.77	5
	5	15	23.64	28.66	5
11%	1	16	21.94	26.32	5
	2	17	25.82	30.89	5
	3	18	22.83	28.13	5
	4	19	24.10	28.72	5
	5	20	22.46	27.38	5
25%	1	21	22.66	27.84	5
	2	22	23.00	26.13	4
	3	23	22.96	27.17	5
	4	24	27.21	31.83	5
	5	25	27.87	30.96	4
50%	1	26	23.20	27.40	5
	2	27	25.26	29.59	5
	3	28	32.01	36.31	5
	4	29 ⊙	12.01	26.77	5
	5	30 ⊙	17.13	21.99	5
100%	1	31 ⊙	18.15	23.38	5
	2	32 ⊙	18.09	22.84	5
	3	33 ⊙	17.31	21.61	5
	4	34 ⊙	17.45	22.96	5
	5	35 ⊙	19.27	24.45	5

	Oven Event 1	Oven Event 2
Oven ID:	Beetle/bub	Beetle/bub
Date/Time/Initials In Oven:	08/25/23, 14:17 TT	8/28/23 16:50 JJ
Oven Temp °C:	111	99.1
② Date/Time/Initials Out Oven into Dessicator:	8/27/23 08:30 NL	8/29/23 16:58 JJ
Date/Time/Initials Weighed:	8/27/23 14:53 TT	8/30/23 12:04 TW
Balance ID:	3	3

① Did not 29-35 made on separate date, went in oven Boats 8/27/23, 15:20, @ 111°C - TT 8/27/23

② 29-35 out of oven into dessicator on 8/28/23, 08:26 LG-LG 8/28

V.4

CLIENT	Alon Asphalt Company	DATE RECEIVED	8/21/23	PROTOCOL	TOX 002
PROJECT	Alon Asphalt Company	TEST START DATE	8/21/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	Outfall #1- Grab	TEST END DATE	8/28/23	SPECIES	<i>Atherinops affinis</i>
LAB SAMPLE ID	P230821.01	MATRIX	Liquid	NO. OF ORGANISMS	5

7 Day Chronic Toxicity with Topsmelt

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	1250.0	1250		
	Salt Control	#VALUE!	#VALUE!	1250		
	3.4%	42.5	1207.5	1250		
	11%	137.5	1112.5	1250		
	25%	312.5	937.5	1250		
	50%	625	625.0	1250		
	100%	1250	0.0	1250		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
1 - 6	0%	0	1000.0	1000
	Salt Control	#VALUE!	#VALUE!	1000
	3.4%	34	966.0	1000
	11%	110	890.0	1000
	25%	250	750.0	1000
	50%	500	500.0	1000
	100%	1000	0.0	1000

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
8/21/23	7	P230821.01	FSW081323.01	LG
8/22/23	7	P230821.01	FSW081323.01	TW
8/23/23	7	P230821.01	FSW082023.01	TW
8/24/23	7	P230823.01	FSW082023.01	II
8/25/23	7	P230823.01	FSW082023.01	II
8/26/23	7	P230825.01	FSW082023.01	II
8/27/23	7	P230825.01	FSW082023.01	II

① I.E. - T 8/27/23

**POWER STANDARD CALCULATIONS**

**Topsmelt Mean Growth per Survivor**

Chronic Power Standard Calculation

	average growth/survivor					
Replicate	1	2	3	4	5	Mean
CCEC (3.4)	0.87	0.998	1.192	1.18	1.004	1.0488
Control	0.952	0.714	1.08	0.9825	0.9225	0.9302

Control Mean - CCEC Mean

-0.1186

Difference Divided by Control Mean

-0.12749946

Express as %

-13%

≤39% meets the power standard

Pass

## ORGANISM RECEIPT LOG

<b>Date:</b> 8/10/23	<b>Time:</b> 1230	<b>Batch No.</b> ABS081823.01					
<b>Organism:</b> <p style="text-align: center; font-size: 1.2em;">Atherinops affinis</p>							
<b>Source / Supplier:</b> <p style="text-align: center; font-size: 1.2em;">Aquatic Biosystems</p>							
<b>No. Ordered:</b> <del>610</del> <sup>①</sup> 560	<b>No. Received:</b> 610	<b>Source Batch:</b> Collection date, hatch date, etc.): Hatch: 8/10/23					
<b>Condition of Organisms:</b> <p style="text-align: center; font-size: 1.2em;">Good</p>		<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): <p style="text-align: center; font-size: 1.2em;">8 days</p>					
<b>Shipper:</b> <p style="text-align: center; font-size: 1.2em;">Fedex</p>		<b>B of L (Tracking No.)</b> <p style="text-align: center; font-size: 1.2em;">137496024119</p>					
<b>Condition of Container:</b> <p style="text-align: center; font-size: 1.2em;">Good</p>		<b>Received By:</b> <p style="text-align: center; font-size: 1.2em;">M. Seibert</p>					
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	10.7	20.9	29 ppt	7.2	20	-	NS
2	10.2	20.9	29 ppt	7.1	20 <span style="border: 1px solid black; border-radius: 50%; padding: 2px;">Total: 40</span>	Total: 7.5%	NS
<small>*if &gt;10% contact lab manager</small>							
<b>Notes:</b>							

① WC-MS 8/10

1300 Blue Spruce Drive, Suite C  
Fort Collins, Colorado 80524



Toll Free: 800/331-5916  
Tel: 970/484-5091 Fax: 970/484-2514

### ORGANISM HISTORY

DATE: 8/17/2023

SPECIES: Atherinops affinis

AGE: 7 day

LIFE STAGE: Larvae

HATCH DATE: 8/10/2023

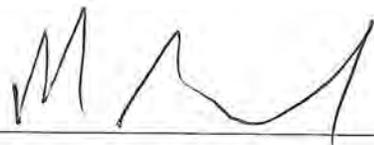
BEGAN FEEDING: Immediately

FOOD: Artemia sp.

### Water Chemistry Record:

	Mean	Range
TEMPERATURE:	<u>19°C</u>	<u>--</u>
SALINITY/CONDUCTIVITY:	<u>30 ppt</u>	<u>--</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>140 mg/l</u>	<u>--</u>
pH:	<u>7.60</u>	<u>--</u>

### Comments:

  
\_\_\_\_\_  
*Facility Supervisor*

## MAINTENANCE LOG FOR CULTURES

ORGANISM: Topples  
 LOCATION: Bath 1

Batch Number: ABS081823.01    Date Received: 8/18/23    Initial # of Organisms: 610    10% mortality = 61

Date	Feed AM/PM	Tub No.	D.O.	Temp (°C)	Cond/ (Sal)	pH	H <sub>2</sub> O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	<sup>1</sup> Cumulative # Mort*	Init.	Comments
8/19	✓ ✓	A	6.8	19.5	30	7.8	Y	Y	0	—	NL	
8/19	✓ ✓	B	6.8	19.5	30	7.8	Y	Y	4	—	NL	
8/19	✓ ✓	C	6.9	19.6	30	7.8	Y	Y	0	4	NL	
8/20	✓ ✓	A	6.9	19.5	30	7.7	Y	Y	10	—	UG	
8/20	✓ ✓	B	7.0	19.5	30	7.8	Y	Y	0	—	UG	
8/20	✓ ✓	C	6.8	19.7	30	7.7	Y	Y	0	14	UG	
8/21	✓ ✓	A	7.0	19.0	31	7.8	N	Y	0	—	TW	
8/21	✓ ✓	B	6.9	19.0	31	7.9	N	Y	0	—	TW	
8/21	✓ ✓	C	7.0	19.2	31	7.8	N	Y	0	14	TW	
8/22	✓	A	7.3	19.1	31	7.9	Y	Y	102	116	TW	condesed A,B,C to A
<del>Finished Culture</del>												

FT = Flow-through

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the current day's total mortality + previous cumulative culture mortality since acquired in lab

8/8/23

Culture Maintenance Log V1.4

## **APPENDIX A2.1**

Reference Toxicant

*Americamysis bahia* (Opossum Shrimp)

48-Hour Acute Test

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Test Type: Survival

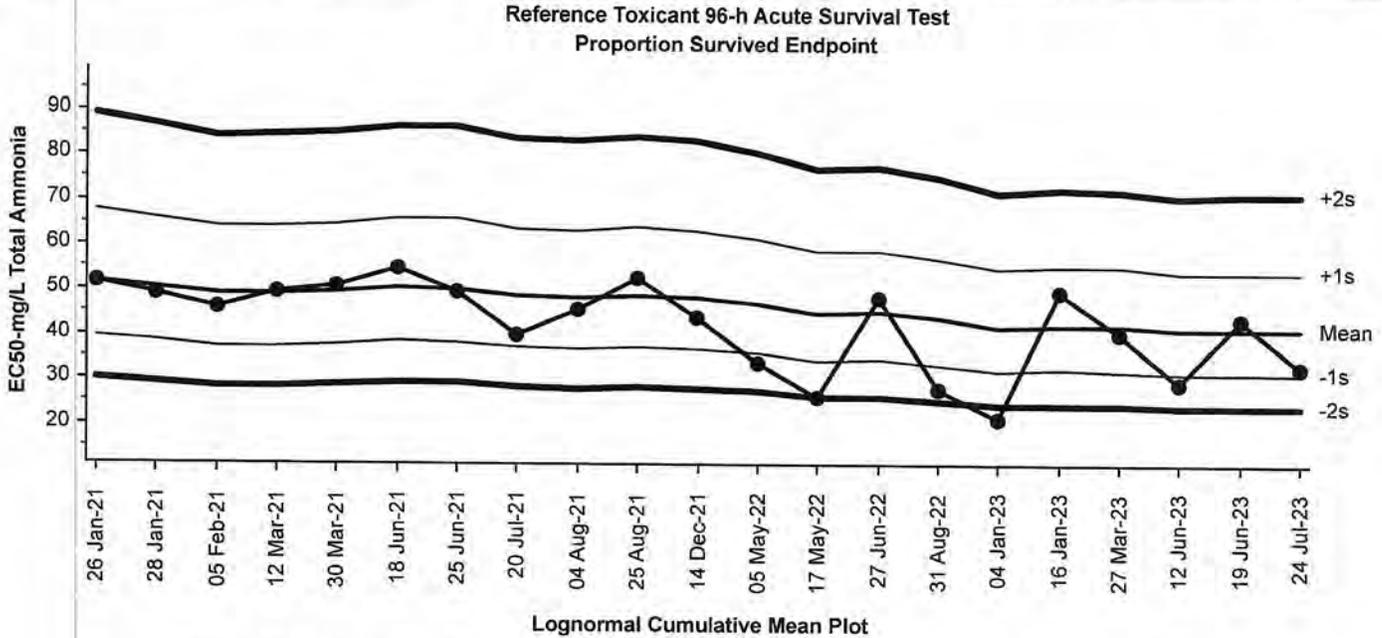
Organism: Americamysis bahia

Material: Total Ammonia

Protocol: EPA/821/R-02-012 (2002)

Endpoint: Proportion Survived

Source: Reference Toxicant-REF



Lognormal Cumulative Mean Plot  
 Mean: 41.26      Count: 20      -1s Warning Limit: 31.4      -2s Action Limit: 24  
 Sigma: NA      CV: 27.70%      +1s Warning Limit: 54.1      +2s Action Limit: 71

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Jan	26	16:52	51.72	10.46	0.8312			10-9780-3692	03-4036-5177
2			28	16:20	48.87	7.601	0.6221			14-7963-0355	07-3515-3787
3		Feb	5	15:08	46.19	4.927	0.415			19-8703-5268	12-3605-4689
4		Mar	12	16:14	49.37	8.103	0.6597			18-5434-9515	18-6079-9086
5			30	13:55	50.83	9.563	0.7669			17-3756-7744	04-0053-5233
6		Jun	18	16:30	54.64	13.37	1.033	(+)		01-8844-2510	20-0148-5495
7			25	14:10	49.31	8.041	0.655			18-5484-8878	12-4713-5568
8		Jul	20	14:00	39.61	-1.65	-0.1501			13-1683-1120	17-0510-9818
9		Aug	4	15:31	45.6	4.34	0.3679			18-5136-2569	14-9688-8086
10			25	12:30	52.66	11.4	0.8973			17-5720-9959	08-5446-9120
11		Dec	14	15:08	43.78	2.514	0.2176			12-2936-9872	13-6771-0841
12	2022	May	5	14:59	33.55	-7.711	-0.7611			20-5665-2087	14-9264-7880
13			17	15:00	25.96	-15.31	-1.705	(-)		09-5954-8213	20-8845-5762
14		Jun	27	15:16	48.24	6.974	0.5745			04-2763-8518	05-6292-1544
15		Aug	31	15:38	28.01	-13.25	-1.425	(-)		09-7289-9321	20-5773-2495
16	2023	Jan	4	16:54	21.02	-20.24	-2.482	(-)	(-)	10-8405-7934	14-3917-7655
17			16	15:20	49.73	8.468	0.6867			00-5023-4163	02-3920-9354
18		Mar	27	16:15	40.05	-1.215	-0.11			19-9864-9381	05-2366-4559
19		Jun	12	18:15	28.97	-12.3	-1.302	(-)		21-2321-1661	20-4768-3034
20			19	16:45	43.42	2.159	0.1876			06-8112-6501	18-4426-3725
21		Jul	24	16:30	32.5	-8.764	-0.8785			02-6642-7447	19-0861-9788

# CETIS Summary Report

Report Date: 31 Aug-23 09:29 (p 1 of 1)  
 Test Code/ID: P220819.72 / 02-6642-7447

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 08-3007-5100      Test Type: Survival      Analyst: Nicole Lundgren  
 Start Date: 24 Jul-23 16:30      Protocol: EPA/821/R-02-012 (2002)      Diluent: Laboratory Seawater  
 Ending Date: 28 Jul-23 15:46      Species: Americamysis bahia      Brine: Not Applicable  
 Test Length: 95h      Taxon: Malacostraca      Source: Aquatic Biosystems, CO      Age: 4d

Sample ID: 07-6047-8161      Code: P220819.72      Project: Reference Toxicant  
 Sample Date: 19 Aug-22      Material: Total Ammonia      Source: Reference Toxicant  
 Receipt Date: 19 Aug-22      CAS (PC):      Station: P220819.72  
 Sample Age: 339d 16h      Client: Internal Lab

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
09-7902-9442	Proportion Survived	Fisher Exact Test	20.1	43.6	29.6	---	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
19-0861-9788	Proportion Survived	Spearman-Kärber	EC50	32.5	30.15	35.04	1

### Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	3	0.9333	0.7899	1.0770	0.9000	1.0000	0.0333	0.0577	6.19%	0.00%
9.23		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-7.14%
20.1		3	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-7.14%
43.6		3	0.1667	0.0232	0.3101	0.1000	0.2000	0.0333	0.0577	34.64%	82.14%
60.1		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
80.4		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### Proportion Survived Detail

MD5: 973A83DAA93C17ED1BF1DCDDA5164C0B

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	1.0000	0.9000	0.9000
9.23		1.0000	1.0000	1.0000
20.1		1.0000	1.0000	1.0000
43.6		0.1000	0.2000	0.2000
60.1		0.0000	0.0000	0.0000
80.4		0.0000	0.0000	0.0000

### Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	10/10	9/10	9/10
9.23		10/10	10/10	10/10
20.1		10/10	10/10	10/10
43.6		1/10	2/10	2/10
60.1		0/10	0/10	0/10
80.4		0/10	0/10	0/10

# CETIS Test Data Worksheet

Report Date: 30 Aug-23 11:59 (p 1 of 1)  
 Test Code/ID: P220819.72 / 02-6642-7447

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Start Date: 24 Jul-23 16:30 Species: Americamysis bahia  
 End Date: 28 Jul-23 15:46 Protocol: EPA/821/R-02-012 (2002)  
 Sample Date: 19 Aug-22 Material: Total Ammonia  
 Sample Code: P220819.72  
 Sample Source: Reference Toxicant  
 Sample Station: P220819.72

Conc-mg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	4	10	10	
0	D	2	11	10	9	
0	D	3	2	10	9	
9.23		1	5	10	10	
9.23		2	15	10	10	
9.23		3	6	10	10	
20.1		1	8	10	10	
20.1		2	12	10	10	
20.1		3	13	10	10	
43.6		1	14	10	1	
43.6		2	17	10	2	
43.6		3	18	10	2	
60.1		1	7	10	0	
60.1		2	16	10	0	
60.1		3	10	10	0	
80.4		1	1	10	0	
80.4		2	3	10	0	
80.4		3	9	10	0	

**96-Hour Mysid Ammonia Reference Toxicant Test**

Toxicant:	Ammonium Chloride
Ref Tox ID:	P220819.72
Lot #:	22E3156086
Protocol:	TOX099
Replicates:	3

Date Test Started:	7/24/2023
Date Test Ended:	7/28/2023
Matrix:	Liquid
Species:	Americamysis bahia
No. of Org. per Chamber:	10

	Conc.	Meter #:	DO (mg/L) (>4.6)	Meter #:	Temp (°C) (20±1°C)	Meter #:	Salinity (ppt) (30±2ppt)	Meter #:	pH (7 - 9)
<b>Day 0 (Stock)</b>	Control	7	7.6	7	20.7	7	31	7	7.5
Date: 7/24/23	10		7.6		20.8		31		7.7
Time: 1410	20		7.7		21.0		31		7.7
Technician: TW	40		7.6		20.7		31		7.6
PM Feed: SE	60		7.7		20.8		31		7.6
	80		7.7		21.1		31		7.5
			Day 1		Day 2		Day 3		Day 4
Temperature (OLD)			20.2		20.5		20.1		—
Temperature (NEW)					19.2				
Feed: 0.1mL Artemia (Time/Init)	AM		LG		TW		SE		NL
	PM		TW		SE		NL		—
<b>Day 4</b>	Control	7	5.2	7	22.1	7	32	7	7.3
Date: 07/28/23	10		5.4		20.8		32		7.6
Time: 1428	20		5.3		20.9		32		7.5
Replicate No.: 3	40		6.3		20.9		32		7.7
Technician: SR	60		—		—		—		—
	80		—		—		—		—

**Dilution Preparation (Mysid NH<sub>3</sub> RT Spiking Worksheet)**

Test Solution NH <sub>3</sub> Concentration			Volume of Stock to Reach Desired Concentration	
Measured	Desired	Volume	mL stock to increase	
mg/L	mg/L	mL	SALT WATER	
Day 0	Day 2			
0	0	750		0.000
9.23	10.9	750		0.920
20.1	21.3	750		1.839
43.6	43.8	750		3.678
60.1	59.9	750		5.518
80.4	76.3	750		7.357

Start Time:	11030 LG, TW, SE, MS
End Time:	1546 SR
Test Acceptability:	✓ ≥90% survival in control

Test Location:	Bath 1
Dilution Water Batch:	ESW072423.01
Supplier:	Aquatic BioSystems
Organism Batch:	AB607223.02
Chamber Size/Type:	12 oz. Cup
Exposure Volume:	250 mL

① IE-MS 7/24

**96-Hour Mysid Ammonia Reference Toxicant Test**

Toxicant:	Ammonium Chloride
Ref Tox ID:	P220819.72
Species:	Americamysis bahia

Date Test Started:	7/24/2023
Date Test Ended:	7/28/2023

Concentration	Rep	Day 1		Day 2		Day 3		Day 4	
		Date: 07/25/23 Time: 1022		Date: 7/26/23 Time: 1320		Date: 7/27/23 Time: 1023		Date: <del>5/07/23/23</del> Time: 1546	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	9	1NB	9	0	9	0	9	0
	3	9	1NB	9	0	9	0	9	0
10	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
20	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
40	1	9	1	6	3	4	2	2	3
	2	8	2NB	4	4	4	0	2	2
	3	10	0	5	3, 2NB	4	1	2	2
60	1	7	3	0	4, 3NB	-	-	/	
	2	9	2	1	7, 1NB	0	1		
	3	7	3	1	4, 2NB	0	1		
80	1	5	5	0	5	/		/	
	2	6	4	0	6				
	3	7	3	0	7				
INITIALS:		SR		TW		NL		SR	

① SM - SR 07/28/23

**Ammonia Reference Toxicant  
Spiking Worksheet**

Reference Toxicant ID: P220819.72  
 Date Prepared: 7/24/23  
 Technician Initials: SZ

# Mysid NH<sub>3</sub> RT

Assumptions in Model  
 Stock ammonia concentration is 9,000 mg/L = 9 mg/mL

Date: 7/6/2023  
 Measurement: 7646.666667

Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	Desired Concentration	Volume		
mg/L	mg/L	mL	mL stock to increase	
0.00	0	750		SALT WATER
9.23	10	750		1.471
20.1	20	750		2.942
43.6	40	750		5.885 → 4.1
60.1	60	750		8.827 → 6.2
80.4	80	750		11.770 → 8.2

1	11
2	10
3	15
4	1
5	12
6	6
7	2
8	4
9	8
10	13
11	18
12	17
13	16
14	5
15	7
16	3
17	9
18	14

Mysid NH3 RT

P#: p220819.72

## **APPENDIX A2.2**

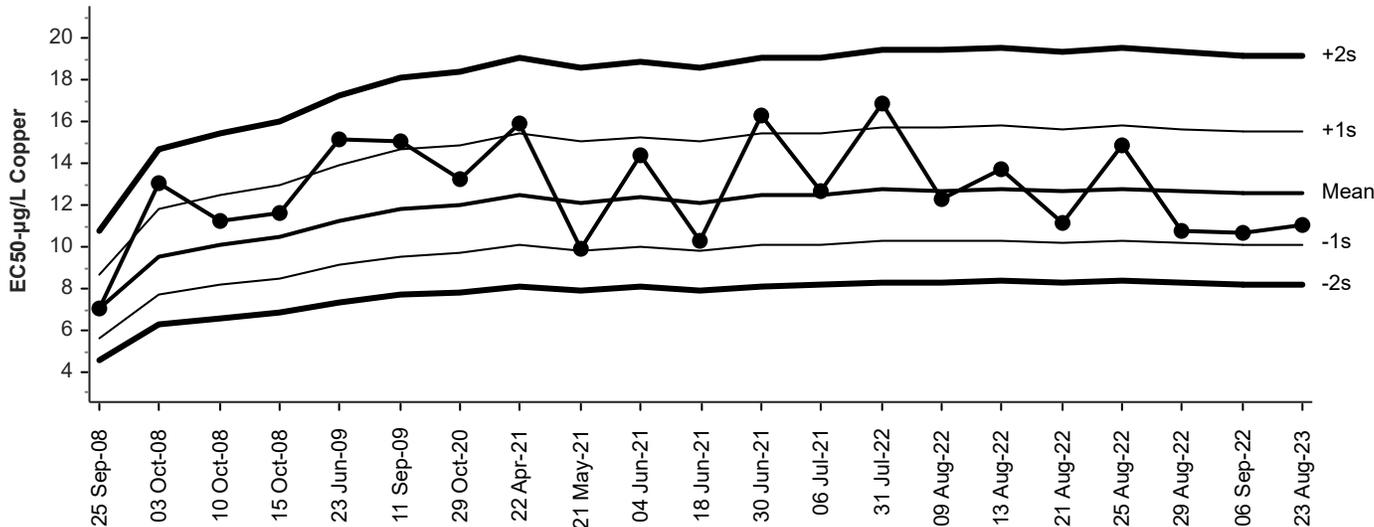
Reference Toxicant

*Dendraster excentricus* (Sand Dollar)

72-Hour Chronic Test

<b>Echinoid Embryo-Larval Development Test</b>			<b>All Matching Labs</b>		
<b>Test Type:</b> Development-Survival	<b>Organism:</b> Dendraster excentricus	<b>Material:</b> Copper			
<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Endpoint:</b> Combined Proportion Normal	<b>Source:</b> Reference Toxicant-REF			

**Echinoid Embryo-Larval Development Test  
Combined Proportion Normal Endpoint**



**Lognormal Cumulative Mean Plot**

<b>Mean:</b> 12.57	<b>Count:</b> 20	<b>-1s Warning Limit:</b> 10.2	<b>-2s Action Limit:</b> 8.23
<b>Sigma:</b> NA	<b>CV:</b> 21.40%	<b>+1s Warning Limit:</b> 15.5	<b>+2s Action Limit:</b> 19.2

**Quality Control Data**

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2008	Sep	25	21:00	7.041	-5.525	-2.737	(-)	(-)	15-9124-4449	18-8842-3071	NewFields
2		Oct	3	18:45	13.07	0.5066	0.1868			11-9535-1619	11-5972-7732	NewFields
3			10	21:45	11.24	-1.331	-0.529			06-3941-0514	03-0947-4870	NewFields
4			15	18:40	11.68	-0.8862	-0.3456			06-1920-3090	16-4174-1254	NewFields
5	2009	Jun	23	16:30	15.18	2.612	0.8926			04-9350-4568	07-0436-8570	NewFields
6		Sep	11	16:45	15.09	2.523	0.8646			06-0487-8749	06-8944-5534	NewFields
7	2020	Oct	29	17:43	13.26	0.6949	0.2544			10-9996-5743	10-4344-5572	EcoAnalysts
8	2021	Apr	22	15:28	15.89	3.32	1.108	(+)		13-0526-7159	09-9497-2946	EcoAnalysts
9		May	21	16:13	9.98	-2.587	-1.089	(-)		18-2275-6252	02-1735-2505	EcoAnalysts
10		Jun	4	14:40	14.41	1.848	0.6486			12-8084-3683	15-1707-5018	EcoAnalysts
11			18	13:31	10.32	-2.247	-0.9313			09-2943-7453	14-7301-3948	EcoAnalysts
12			30	14:34	16.3	3.73	1.228	(+)		13-1533-7314	11-9286-9645	EcoAnalysts
13		Jul	6	16:32	12.65	0.0847	0.03175			08-9622-1833	16-9139-3087	EcoAnalysts
14	2022		31	12:04	16.85	4.282	1.386	(+)		17-1430-0812	08-1823-5836	EcoAnalysts
15		Aug	9	13:52	12.29	-0.2766	-0.1052			12-9511-5607	07-0707-5135	EcoAnalysts
16			13	14:57	13.77	1.204	0.4324			12-4958-8455	07-2397-4153	EcoAnalysts
17			21	11:53	11.12	-1.442	-0.576			09-6948-7001	11-1529-5767	EcoAnalysts
18			25	14:27	14.9	2.336	0.8059			04-4887-1465	12-9984-0141	EcoAnalysts
19			29	13:35	10.74	-1.824	-0.7412			09-1917-8280	10-2102-8777	EcoAnalysts
20		Sep	6	12:45	10.66	-1.904	-0.7766			02-6918-0642	01-6664-9544	EcoAnalysts
21	2023	Aug	23	15:07	11.1	-1.469	-0.5874			05-1315-2824	15-1986-4286	EcoAnalysts

# CETIS Summary Report

Report Date: 19 Sep-23 13:56 (p 1 of 3)  
 Test Code/ID: P220110.106 / 05-1315-2824

## Echinoid Embryo-Larval Development Test

EcoAnalysts

Batch ID: 19-2984-3037	Test Type: Development-Survival	Analyst: Marisa Seibert
Start Date: 23 Aug-23 15:07	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 26 Aug-23 16:38	Species: Dendraster excentricus	Brine: Crystal Sea Marine Mix
Test Length: 74h	Taxon: Echinoidea	Source: In-House Culture
Sample ID: 13-0383-4529	Code: P220110.106	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.106
Sample Age: 590d 15h	Client: Internal Lab	Age:

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
01-3146-3495	Combined Proportion Normal	Dunnett Multiple Comparison Test	✓ 7.5	15	10.61	7.48%	1
08-7836-5893	Proportion Normal	Dunnett Multiple Comparison Test	✓ 7.5	15	10.61	2.83%	1
16-2679-9439	Proportion Survived	Steel Many-One Rank Sum Test	60	>60	---	2.9%	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
15-1986-4286	Combined Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	8.449	8.34	8.494	1
			EC20	8.789	8.68	8.851	
			EC25	9.14	9.02	9.221	
			EC40	10.27	10.11	10.42	
			EC50	11.1	10.91	11.3	
02-8119-8933	Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	8.444	8.318	8.483	1
			✓ EC20	8.781	8.647	8.835	
			✓ EC25	9.13	8.996	9.2	
			✓ EC40	10.25	10.09	10.38	
			✓ EC50	11.07	10.88	11.24	
18-4683-1422	Proportion Survived	Linear Interpolation (ICPIN)	EC15	>60	---	---	1
			EC20	>60	---	---	
			EC25	>60	---	---	
			EC40	>60	---	---	
			EC50	>60	---	---	

# CETIS Summary Report

Report Date: 19 Sep-23 13:56 (p 2 of 3)  
 Test Code/ID: P220110.106 / 05-1315-2824

## Echinoid Embryo-Larval Development Test

EcoAnalysts

### Combined Proportion Normal Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9588	0.9145	1.0030	0.9400	1.0000	0.0139	0.0278	2.90%	0.00%
3.75		4	0.9775	0.9160	1.0390	0.9200	1.0000	0.0193	0.0386	3.95%	-1.96%
7.5		4	0.9862	0.9425	1.0300	0.9450	1.0000	0.0138	0.0275	2.79%	-2.87%
15		4	0.1012	0.0483	0.1542	0.0800	0.1500	0.0166	0.0333	32.85%	89.44%
30		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
60		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### Proportion Normal Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9642	0.9451	0.9832	0.9543	0.9793	0.0060	0.0120	1.24%	0.00%
3.75		4	0.9559	0.9326	0.9792	0.9340	0.9635	0.0073	0.0146	1.53%	0.86%
7.5		4	0.9744	0.9568	0.9920	0.9615	0.9859	0.0055	0.0111	1.14%	-1.06%
15		4	0.0960	0.0415	0.1506	0.0693	0.1463	0.0172	0.0343	35.72%	90.04%
30		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
60		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9862	0.9616	1.0110	0.9650	1.0000	0.0077	0.0155	1.57%	0.00%
3.75		4	0.9888	0.9529	1.0250	0.9550	1.0000	0.0113	0.0225	2.28%	-0.25%
7.5		4	0.9938	0.9739	1.0140	0.9750	1.0000	0.0063	0.0125	1.26%	-0.76%
15		4	0.9912	0.9634	1.0190	0.9650	1.0000	0.0088	0.0175	1.77%	-0.51%
30		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.39%
60		4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	-1.39%

### Combined Proportion Normal Detail

MD5: 2F57312C9895F9757BAEEA97752374FF

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9450	0.9500	0.9400	1.0000
3.75		1.0000	0.9200	1.0000	0.9900
7.5		1.0000	1.0000	0.9450	1.0000
15		0.0950	0.1500	0.0800	0.0800
30		0.0000	0.0000	0.0000	0.0000
60		0.0000	0.0000	0.0000	0.0000

### Proportion Normal Detail

MD5: 1F714C89DA48C446DF23D8FF5102B9DB

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9793	0.9548	0.9543	0.9683
3.75		0.9628	0.9634	0.9635	0.9340
7.5		0.9859	0.9810	0.9692	0.9615
15		0.0856	0.1463	0.0829	0.0693
30		0.0000	0.0000	0.0000	0.0000
60		0.0000	0.0000	0.0000	0.0000

### Proportion Survived Detail

MD5: 2C9E45D41BFB90C303801927374C90A4

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9650	0.9950	0.9850	1.0000
3.75		1.0000	0.9550	1.0000	1.0000
7.5		1.0000	1.0000	0.9750	1.0000
15		1.0000	1.0000	0.9650	1.0000
30		1.0000	1.0000	1.0000	1.0000
60		1.0000	1.0000	1.0000	1.0000

# CETIS Summary Report

Report Date: 19 Sep-23 13:56 (p 3 of 3)

Test Code/ID: P220110.106 / 05-1315-2824

## Echinoid Embryo-Larval Development Test

EcoAnalysts

### Combined Proportion Normal Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	189/200	190/200	188/200	214/214
3.75		207/207	184/200	211/211	198/200
7.5		210/210	206/206	189/200	250/250
15		19/200	30/200	16/200	16/200
30		0/200	0/200	0/200	0/200
60		0/200	0/200	0/200	0/200

### Proportion Normal Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	189/193	190/199	188/197	214/221
3.75		207/215	184/191	211/219	198/212
7.5		210/213	206/210	189/195	250/260
15		19/222	30/205	16/193	16/231
30		0/247	0/235	0/221	0/220
60		0/274	0/228	0/250	0/223

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	193/200	199/200	197/200	200/200
3.75		200/200	191/200	200/200	200/200
7.5		200/200	200/200	195/200	200/200
15		200/200	200/200	193/200	200/200
30		200/200	200/200	200/200	200/200
60		200/200	200/200	200/200	200/200

**CETIS Test Data Worksheet**

Report Date: 19 Sep-23 13:55 (p 1 of 1)  
 Test Code/ID: P220110.106 / 05-1315-2824

<b>Echinoid Embryo-Larval Development Test</b>					<b>EcoAnalysts</b>
<b>Start Date:</b> 23 Aug-23 15:07	<b>Species:</b> Dendroaster excentricus	<b>Sample Code:</b> P220110.106			
<b>End Date:</b> 26 Aug-23 16:38	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Sample Source:</b> Reference Toxicant			
<b>Sample Date:</b> 10 Jan-22	<b>Material:</b> Copper	<b>Sample Station:</b> P220110.106			

Conc-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	22	200	193	193	189	
0	D	2	17	200	199	199	190	
0	D	3	8	200	197	197	188	
0	D	4	5	200	221	221	214	
3.75		1	19	200	215	215	207	
3.75		2	14	200	191	191	184	
3.75		3	12	200	219	219	211	
3.75		4	21	200	212	212	198	
7.5		1	20	200	213	213	210	
7.5		2	2	200	210	210	206	
7.5		3	15	200	195	195	189	
7.5		4	18	200	260	260	250	
15		1	7	200	222	222	19	
15		2	9	200	205	205	30	
15		3	4	200	193	193	16	
15		4	23	200	231	231	16	
30		1	11	200	247	247	0	
30		2	10	200	235	235	0	
30		3	16	200	221	221	0	
30		4	6	200	220	220	0	
60		1	3	200	274	274	0	
60		2	24	200	228	228	0	
60		3	1	200	250	250	0	
60		4	13	200	223	223	0	

**96 Hour Echinoderm Development Reference Toxicant Test**

Test ID: P220110.104	Replicates: 3	Study Director: M. Seibert	Location: Inc. 1
Dilution Water Batch: FSW082323.01	Organism Batch: De081023.01	Associated Test(s): Alon Asphalt Co.	Organism: Dendroaster excentricus
Chamber Size/Type: 30 ml shell vial	Exposure Volume: 10 ml		
Toxicant: Copper Chloride (400,000 µg Cu/L CuCl <sub>2</sub> )	Lot #: MKCK7155	Date Prepared: 8/23/23	Initials: NL TW
Target Concentrations: 60 µg/L	Quantity of Stock Target: 75 µL	Quantity of Diluent Target: 500 mL	

Serial dilute by 50%

<b>0 Hours</b>	Date: 8/23/23	WQ Time: 1203	Start Time: 1507	Initials: NL, VI
----------------	------------------	------------------	---------------------	---------------------

**STOCK**

	Control	3.75	7.5	15	30	60
D.O. (%) (>4.0 mg/L)	7.5	7.6	7.6	7.7	7.7	7.8
Temperature (15 ± 1°C) ②	15.5	15.5	15.5	15.5	15.5	15.5
Salinity (34 ± 2 ppt EPA) (30 ± 2 ppt WDOE)	30	30	31	31	31	31
pH (6-9)	7.8	7.9	7.9	7.9	7.9	7.9

<b>Day 1</b>	Temperature (15 ± 1°C)	14.2	14.2			
--------------	---------------------------	------	------	--	--	--

<b>Day 2</b>	Temperature (15 ± 1°C)	14.3	NL	T21		
--------------	---------------------------	------	----	-----	--	--

<b>Day 3</b>	Temperature (15 ± 1°C)	14.5	NL	T14		
--------------	---------------------------	------	----	-----	--	--

Day 3

<b>Day 4</b>	Date: 8/26	WQ Time: 1645	End Time: 1638	Initials: NL
--------------	------------	---------------	----------------	--------------

	Formalin Lot #: 220304-50	Rose Bengal Lot #: 5135		
--	------------------------------	----------------------------	--	--

**STOCK**

	Control	3.75	7.5	15	30	60
D.O. (%) (>4.0 mg/L)	8.2	8.2	8.2	8.2	8.1	8.2
Temperature (15 ± 1°C) ②	14.5	14.5	14.5	14.5	14.5	14.5
Salinity (34 ± 2 ppt EPA) (30 ± 2 ppt WDOE)	31	31	31	31	31	31
pH (6-9)	8.0	8.0	8.0	8.0	8.0	8.0

Notes: ① IE-NL 8/23/23  
② Temp blank used - NL 8/23/23, NL 8/26

**96 Hour Echinoderm Development Reference Toxicant Test**

Ref Tox ID: P226110-104

SPAWNING DATA				
Initial Spawning Time: 1325	Final Spawning Time: 1405	Fertilization Time: 1410	No. of Females: 3	No. of Males: 4
Embryo Density (count/mL):	1. 32	2. 37	3. —	Mean: 34.5
Stocking Volume Calculation: $\frac{2700}{3450} = 0.782 \times 100 \text{ mL} = 78.2 \text{ mL egg stock} : 21.7 \text{ mL F/W}$				

Deliver 100 mL

ZERO TIME COUNTS					
1. 211	2. 196	3. 209	4. 188	5. 193	6. 205
Technician: MK <span style="margin-left: 100px;">mean = 200</span>					

LARVAL COUNT DATA									
Conc.	Rep 1		Rep 2		Rep 3		Rep 4		Initials
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	
Control	189	4	190	9	188	9	214	7	MK
3.75	207	8	184	7	211	8	198	14	MARU
7.5	210	3	206	4	189	6	250	10	MARU
15	19	203	30	175	16	177	16	215	MARU
30	0	247	0	235	0	221	0	220	MARU
60	0	274	0	228	0	250	0	223	MARU

QA Count Checks:			
#1 conc/rep 02	#2 conc/rep 3.75 <sub>3</sub>	#3 conc/rep _____	#4 conc/rep _____
# normal 189 # abnormal 9	# normal 207 # abnormal 9	# normal _____ # abnormal _____	# normal _____ # abnormal _____
Tech. Init. DM	Tech. Init. DM	Tech. Init. _____	Tech. Init. _____
Calc. O.G. $\frac{190 \text{ N}}{9 \text{ A}} = 95.5\%$	Calc. O.G. $\frac{211}{219} = 96.3\%$		
QA: $\frac{189}{197} = 95.9\%$	QA: $\frac{207}{216} = 95.8\%$		
$\Delta\% = 0.4\%$	$\Delta\% = 0.5\%$		
QA Check Acceptability: <input checked="" type="checkbox"/> <5% difference in means of QA & orig. counts			

	1	23
	2	24
0	3	15
	4	17
	5	22
3.75	6	13
	7	5
	8	10
	9	21
7.5	10	8
	11	6
	12	9
	13	14
15	14	19
	15	18
	16	3
	17	16
30	18	11
	19	7
	20	2
60	21	1
	22	4
	23	12
	24	20

echinoderm CuCl<sub>2</sub> RT

p#: P220116.10p

## **APPENDIX A2.3**

Reference Toxicant  
*Atherinops affinis* (Topsmelt)  
96-Hour Acute Test

Reference Toxicant 96-h Acute Survival Test

All Matching Labs

Test Type: Survival

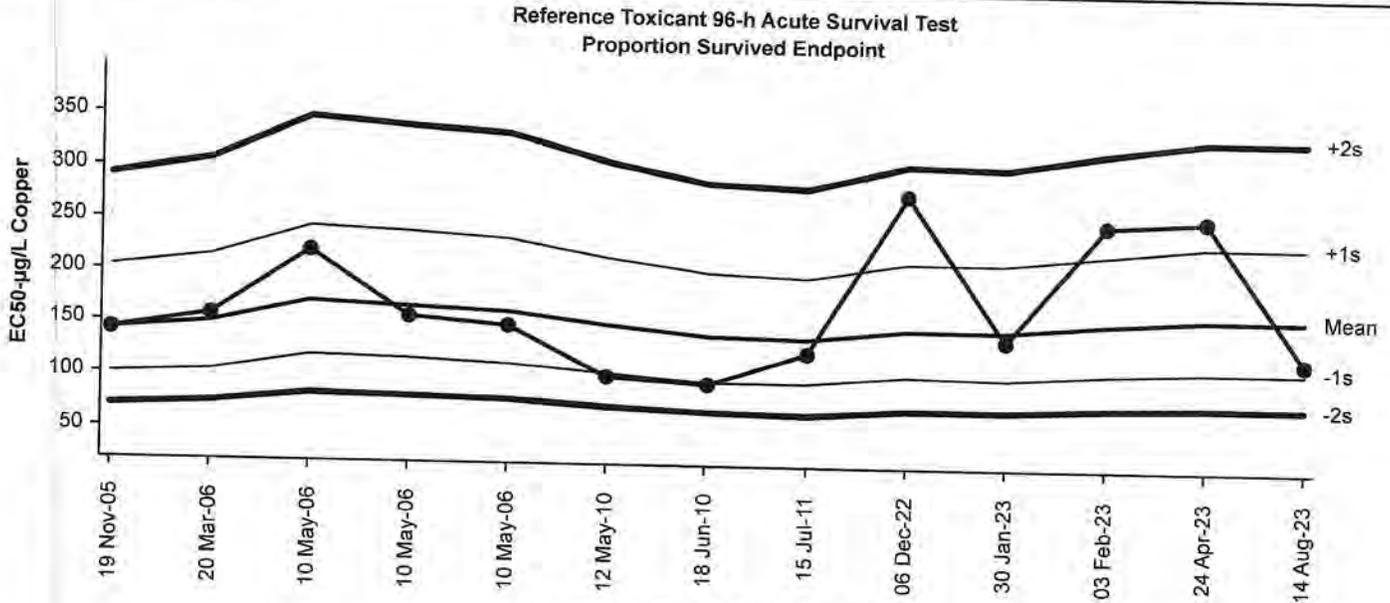
Organism: *Atherinops affinis*

Material: Copper

Protocol: EPA/821/R-02-012 (2002)

Endpoint: Proportion Survived

Source: Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean: 164.2      Count: 12      -1s Warning Limit: 115      -2s Action Limit: 80.4  
 Sigma: NA      CV: 36.90%      +1s Warning Limit: 235      +2s Action Limit: 336

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2005	Nov	19	17:00	142.8	-21.35	-0.39			00-8907-0202	13-7510-8588	NewFields
2	2006	Mar	20	17:00	157.7	-6.5	-0.1131			08-1032-6951	04-8792-0020	NewFields
3		May	10	14:35	220.5	56.35	0.8261			18-7937-4776	08-8928-5451	NewFields
4			10	15:00	158.3	-5.884	-0.1022			01-1060-6502	12-3445-4277	NewFields
5			10	15:15	150.3	-13.9	-0.2477			02-4919-0013	21-1728-8213	NewFields
6	2010		12	16:00	101.7	-62.44	-1.34	(-)		10-7151-7987	02-7426-1392	NewFields
7		Jun	18	14:50	96.59	-67.59	-1.485	(-)		07-7473-5259	01-6255-1954	NewFields
8	2011	Jul	15	15:35	126.8	-37.43	-0.7242			18-0945-8408	17-6259-8446	NewFields
9	2022	Dec	6	17:30	280.4	116.3	1.499	(+)		08-8955-3517	18-3778-5416	EcoAnalysts
10	2023	Jan	30	14:21	141.4	-22.76	-0.4179			20-9187-4577	07-5442-1816	EcoAnalysts
11		Feb	3	14:14	254.2	90.05	1.224	(+)		01-8434-2194	04-1023-9110	EcoAnalysts
12		Apr	24	13:02	259.4	95.18	1.28	(+)		15-8288-3680	19-9438-7252	EcoAnalysts
13		Aug	14	16:09	123.7	-40.48	-0.7926			18-5669-0071	21-0532-5790	EcoAnalysts

# CETIS Summary Report

Report Date: 22 Aug-23 10:56 (p 1 of 1)  
 Test Code/ID: P220110.104 / 18-5669-0071

## Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID: 08-7439-3835	Test Type: Survival	Analyst: Marisa Seibert
Start Date: 14 Aug-23 16:09	Protocol: EPA/821/R-02-012 (2002)	Diluent: Laboratory Seawater
Ending Date: 18 Aug-23 14:12	Species: Atherinops affinis	Brine: Not Applicable
Test Length: 94h	Taxon: Actinopterygii	Source: Aquatic Biosystems, CO Age: 14 d
Sample ID: 10-6648-3031	Code: P220110.104	Project: Reference Toxicant
Sample Date: 10 Jan-22	Material: Copper	Source: Reference Toxicant
Receipt Date: 10 Jan-22	CAS (PC):	Station: P220110.104
Sample Age: 581d 16h	Client: Internal Lab	

### Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
02-5845-1986	Proportion Survived	Steel Many-One Rank Sum Test	100	200	141.4	17.3%	1

### Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
21-0532-5790	Proportion Survived	GLM: Log-Normal (Probit)	EC5	53.93	30.73	72.59	1
			EC10	64.78	40.25	83.86	
			EC15	73.31	48.2	92.58	
			EC20	80.89	55.56	100.3	
			EC25	88.01	62.67	107.6	
			EC40	108.9	84.18	129.4	
			EC50	123.7	99.61	146	

### Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9250	0.6863	1.1640	0.7000	1.0000	0.0750	0.1500	16.22%	0.00%
50		4	0.9250	0.7727	1.0770	0.8000	1.0000	0.0479	0.0957	10.35%	0.00%
100		4	0.5750	0.3363	0.8137	0.4000	0.7000	0.0750	0.1500	26.09%	37.84%
200		4	0.2000	0.2000	0.2000	0.2000	0.2000	0.0000	0.0000	0.00%	78.38%
400		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
800		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

### Proportion Survived Detail

MD5: 1E92354EBEB283329D29D685600D221C

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	0.7000	1.0000
50		0.9000	0.8000	1.0000	1.0000
100		0.7000	0.4000	0.7000	0.5000
200		0.2000	0.2000	0.2000	0.2000
400		0.0000	0.0000	0.0000	0.0000
800		0.0000	0.0000	0.0000	0.0000

### Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	7/10	10/10
50		9/10	8/10	10/10	10/10
100		7/10	4/10	7/10	5/10
200		2/10	2/10	2/10	2/10
400		0/10	0/10	0/10	0/10
800		0/10	0/10	0/10	0/10

**CETIS Test Data Worksheet**

Report Date: 22 Aug-23 10:52 (p 1 of 1)  
 Test Code/ID: P220110.104 / 18-5669-0071

<b>Reference Toxicant 96-h Acute Survival Test</b>				<b>EcoAnalysts</b>
<b>Start Date:</b> 14 Aug-23 16:09	<b>Species:</b> Atherinops affinis	<b>Sample Code:</b> P220110.104		
<b>End Date:</b> 18 Aug-23 14:12	<b>Protocol:</b> EPA/821/R-02-012 (2002)	<b>Sample Source:</b> Reference Toxicant		
<b>Sample Date:</b> 10 Jan-22	<b>Material:</b> Copper	<b>Sample Station:</b> P220110.104		

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	21	10	10	
0	D	2	2	10	10	
0	D	3	8	10	7	
0	D	4	6	10	10	
50		1	18	10	9	
50		2	20	10	8	
50		3	19	10	10	
50		4	23	10	10	
100		1	14	10	7	
100		2	10	10	4	
100		3	3	10	7	
100		4	11	10	5	
200		1	9	10	2	
200		2	15	10	2	
200		3	4	10	2	
200		4	16	10	2	
400		1	13	10	0	
400		2	17	10	0	
400		3	12	10	0	
400		4	7	10	0	
800		1	24	10	0	
800		2	22	10	0	
800		3	5	10	0	
800		4	1	10	0	



## 96-Hour Topsmelt CuCl<sub>2</sub> Reference Toxicant Test

Toxicant:	Copper Chloride
Ref Tox ID:	P20110.104
Species:	<i>Atherinops affinis</i>

Date Test Started:	8/15/23
Date Test Ended:	8/18/23

Concentration (µg/L)	Rep	Day 1		Day 2		Day 3		Day 4	
		Date: 8/15/23		Date: 8/16/23		Date: 8/17/23		Date: 8/18/23	
		Time: 0959		Time: 1130		Time: 1059		Time: <del>1300</del> 1412	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	7	3	7	0	7	0	7	0
	4	10	0	10	0	10	0	10	0
50	1	10	0	9	1	9	0	9	0
	2	10	0	10	0	9	1	8	1
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
100	1	8	2	8	0	7	1	7	0
	2	10	0	8	2	7	1	4	3
	3	10	0	9	1	8	1	7	1
	4	9	1	8	1	5	3	5	0
200	1	6	4	4	2	4	0	2	2
	2	7	3	6	1	<del>8</del> 4	<del>8</del> 2	2	3
	3	4	6	4	0	3	1	2	1
	4	5	5	5	0	4	1	2	2
400	1	1	9	0	1	—	—	—	—
	2	1	9	0	1	—	—	—	—
	3	2	8	0	2	—	—	—	—
	4	0	10	—	—	—	—	—	—
800	1	0	10	—	—	—	—	—	—
	2	0	10	—	—	—	—	—	—
	3	0	10	—	—	—	—	—	—
	4	0	10	—	—	—	—	—	—
INITIALS:		J1		TW		NL		J1	

① I.E. TW 8/16/23, NL 8/17  
 I.E. J1 8/18/23 ②

② I.E. - Actual: Alive 5, Dead: 1 - NL 8/17/23

1	4
2	2
3	13
4	12
5	14
6	1
7	6
8	20
9	18
10	7
11	8
12	22
13	3
14	17
15	21
16	23
17	10
18	15
19	19
20	24
21	9
22	5
23	11
24	16

P# P220110.104

Topsmelt CuCl<sub>2</sub> RT Acute  
8/14/23 - 8/18/23

## ORGANISM RECEIPT LOG

<b>Date:</b> 8/11/23		<b>Time:</b> 1235		<b>Batch No.</b> ABS081123.017 <sup>①</sup>			
<b>Organism:</b> Atherinops affinis							
<b>Source / Supplier:</b> ABS							
<b>No. Ordered:</b> 1020		<b>No. Received:</b> 1120		<b>Source Batch:</b> Collection date, <u>hatch date</u> , etc.): 7/31/2023			
<b>Condition of Organisms:</b> good				<b>Approximate Size or Age:</b> (Days from hatch, life stage, size class, etc.): 11 days			
<b>Shipper:</b> Fedex				<b>B of L (Tracking No.):</b> 13749602 3720			
<b>Condition of Container:</b> good				<b>Received By:</b> NL/TT/JI			
Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
A	8.3	22.4	29 ppt	7.0	33	②	J1
B	10.20	22.5	29 ppt	6.92	110	②	TT
C	8.3	22.2	29 ppt	6.9	19	②	NL
*if >10% contact lab manager							
<b>Notes:</b>							

① sm. J1 8/11/23

7/27/15

② more than 10% dead upon arrival  
management notified - NL 8/11/23

Organism Receipt Log v1.1

Page 1 of 1

1300 Blue Spruce Drive, Suite C  
Fort Collins, Colorado 80524



Toll Free: 800/331-5916  
Tel: 970/484-5091 Fax: 970/484-2514

### ORGANISM HISTORY

DATE: 8/10/2023

SPECIES: Atherinops affinis

AGE: 10 day

LIFE STAGE: Larvae

HATCH DATE: 7/31/2023

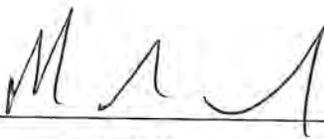
BEGAN FEEDING: Immediately

FOOD: Artemia sp.

### Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>18°C</u>	<u>16-18°C</u>
SALINITY/CONDUCTIVITY:	<u>30 ppt</u>	<u>28-32 ppt</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>140 mg/l</u>	<u>140-200 mg/l</u>
pH:	<u>7.60</u>	<u>7.60-8.10</u>

### Comments:

  
\_\_\_\_\_  
Facility Supervisor



### MAINTENANCE LOG FOR CULTURES

ORGANISM: Toppies

LOCATION: Bath 2

Batch Number: <u>ABS081123.01</u>	Date Received: <u>8/11/23</u>	Initial # of Organisms: <u>1120</u>	10% mortality = <u>112</u>
-----------------------------------	-------------------------------	-------------------------------------	----------------------------

Date	Feed AM/PM	Tub No.	D.O.	Temp (°C)	Cond/ (Sal)	pH	H <sub>2</sub> O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	<sup>1</sup> Cumulative # Mort*	Init.	Comments
8/12	✓ ✓	A	7.1	19.4	29	7.7	Y	Y	8	-	NL	14% dead upon arrival 8/11/23 -NL8/12/23 ↳ a couple bags of topsmelt were squished by other bags -NL8/12/23
8/12	✓ ✓	B	6.9	19.7	29	7.8	Y	Y	7	-	NL	
8/12	✓ ✓	C	6.9	19.8	29	7.7	Y	Y	8	-	NL	
8/12	✓ ✓	D	6.7	20.1	29	7.7	Y	Y	3	-	NL	
8/12	✓ ✓	E	6.7	20.0	29	7.8	Y	Y	13	-	NL	
8/12	✓ ✓	F	6.8	20.0	29	7.8	Y	Y	7	46	NL	
8/13	✓ ✓	A	7.2	19.0	29	7.7	Y	Y	11	-	NL	
8/13	✓ ✓	B	7.1	19.1	29	7.8	Y	Y	1	-	NL	
8/13	✓ ✓	C	7.0	19.3	29	7.8	Y	Y	7	-	NL	
8/13	✓ ✓	D	6.9	19.4	29	7.7	Y	Y	4	-	NL	
8/13	✓ ✓	E	6.9	19.4	29	7.8	Y	Y	11	-	NL	
8/13	✓ ✓	F	6.9	19.4	29	7.8	Y	Y	5	85	NL	
8/14	✓ ✓	A	6.7	18.6	29	7.7	N	Y	2	-	UG	
8/14	✓ ✓	B	7.0	18.7	29	7.8	N	Y	0	-	UG	
8/14	✓ ✓	C	7.0	18.7	29	7.8	N	Y	2	-	UG	
8/14	✓ ✓	D	7.0	18.8	29	7.8	N	Y	0	-	UG	
8/14	✓ ✓	E	7.0	18.9	29	7.8	N	Y	4	-	UG	
8/14	✓ ✓	F	7.2	19.0	29	7.8	N	Y	1	94	UG	

FT = Flow-through

\*For all containers and all days for a given batch; if >10% notify lab manager

<sup>1</sup> Cumulative # Mort is the running total of the current day's total mortality + previous cumulative culture mortality since acquired in lab

## **APPENDIX A2.4**

Reference Toxicant

*Atherinops affinis* (Topsmelt)

7-Day Chronic Test

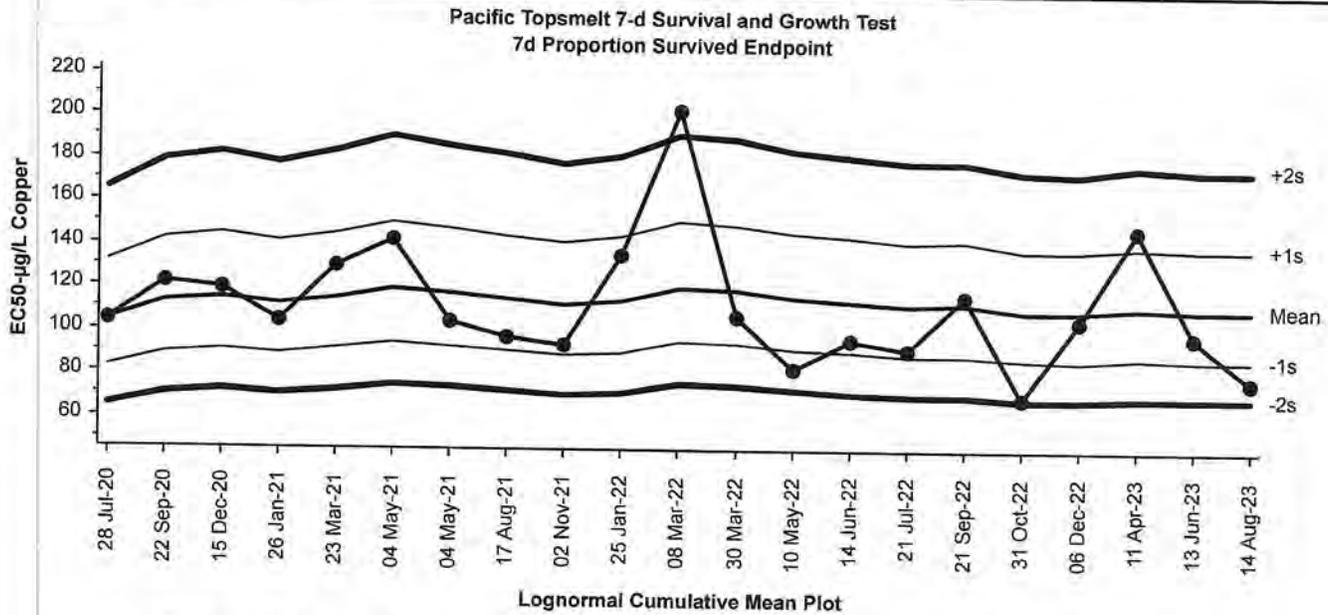
Pacific Topsmelt 7-d Survival and Growth Test

All Matching Labs

Test Type: Growth-Survival (7d)  
 Protocol: EPA/600/R-95/136 (1995)

Organism: Atherinops affinis  
 Endpoint: 7d Proportion Survived

Material: Copper  
 Source: Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean: 110.4      Count: 20      -1s Warning Limit: 87.5      -2s Action Limit: 69.3  
 Sigma: NA      CV: 23.60%      +1s Warning Limit: 139      +2s Action Limit: 176

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2020	Jul	28	16:00	104.1	-6.281	-0.2517			14-0144-0053	09-7965-4210	EcoAnalysts
2		Sep	22	16:11	121.9	11.52	0.4265			00-4076-0952	20-7772-2356	EcoAnalysts
3		Dec	15	16:31	119	8.577	0.3216			02-1504-6456	06-3581-5524	EcoAnalysts
4	2021	Jan	26	15:44	103.7	-6.641	-0.2666			09-7279-9629	04-4959-8826	EcoAnalysts
5		Mar	23	15:37	130	19.63	0.7033			20-9800-6970	10-7439-4071	EcoAnalysts
6		May	4	12:50	142.9	32.5	1.109	(+)		19-6922-3129	19-0566-9610	EcoAnalysts
7			4	13:00	103.9	-6.457	-0.259			00-2283-9750	18-5475-8618	EcoAnalysts
8		Aug	17	19:07	97.27	-13.12	-0.5437			09-0043-0470	14-6071-0348	EcoAnalysts
9		Nov	2	16:06	93.18	-17.21	-0.7283			14-6437-8110	20-9597-1265	EcoAnalysts
10	2022	Jan	25	16:44	135	24.61	0.8649			06-0817-6358	16-0044-1200	EcoAnalysts
11		Mar	8	14:43	202.7	92.31	2.612	(+)	(+)	07-6546-0140	03-2149-8422	EcoAnalysts
12			30	13:20	106.5	-3.907	-0.1549			17-8194-4154	17-1422-7340	EcoAnalysts
13		May	10	16:20	82.35	-28.04	-1.259	(-)		13-9380-0579	14-9529-7356	EcoAnalysts
14		Jun	14	16:08	96.48	-13.91	-0.5786			04-7024-7990	02-4116-4567	EcoAnalysts
15		Jul	21	13:33	91.39	-19	-0.8116			20-8735-8279	00-5003-0764	EcoAnalysts
16		Sep	21	11:00	116.5	6.125	0.2321			01-2995-1270	08-3404-2648	EcoAnalysts
17		Oct	31	16:27	69.85	-40.53	-1.966	(-)		20-9804-3448	17-4046-8765	EcoAnalysts
18		Dec	6	16:28	105.5	-4.929	-0.1963			14-7580-4802	07-9899-2754	EcoAnalysts
19	2023	Apr	11	14:46	147.7	37.34	1.252	(+)		03-7518-1407	18-0066-2550	EcoAnalysts
20		Jun	13	16:35	97.97	-12.42	-0.5129			01-8019-5972	12-3754-2314	EcoAnalysts
21		Aug	14	15:25	77.52	-32.87	-1.519	(-)		06-6644-4095	01-7825-3093	EcoAnalysts



# CETIS Summary Report

Report Date: 27 Sep-23 00:52 (p 1 of 3)  
 Test Code/ID: P220110.103 / 06-6644-4095

## Pacific Topsmelt 7-d Survival and Growth Test

EcoAnalysts

<b>Batch ID:</b> 12-7197-7578	<b>Test Type:</b> Growth-Survival (7d)	<b>Analyst:</b> Marisa Seibert
<b>Start Date:</b> 14 Aug-23 15:25	<b>Protocol:</b> EPA/600/R-95/136 (1995)	<b>Diluent:</b> Laboratory Seawater
<b>Ending Date:</b> 21 Aug-23 13:50	<b>Species:</b> Atherinops affinis	<b>Brine:</b> Not Applicable
<b>Test Length:</b> 6d 22h	<b>Taxon:</b> Actinopterygii	<b>Source:</b> Aquatic Biosystems, CO <b>Age:</b> 14d
<b>Sample ID:</b> 00-4400-5875	<b>Code:</b> P220110.103	<b>Project:</b> Reference Toxicant
<b>Sample Date:</b> 10 Jan-22	<b>Material:</b> Copper	<b>Source:</b> Reference Toxicant
<b>Receipt Date:</b> 10 Jan-22	<b>CAS (PC):</b>	<b>Station:</b> P220110.103
<b>Sample Age:</b> 581d 15h	<b>Client:</b> Internal Lab	

## Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
01-0574-3150	7d Proportion Survived	Dunnett Multiple Comparison Test	✓ 40	80	56.57	34.4%	1
17-8602-2611	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test	✓ 40	80	56.57	29.9%	1
01-7955-6099	Mean Dry Weight-mg	Wilcoxon/Bonferroni Adj Test	✓ 40	80	56.57	29.8%	1

## Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
01-7825-3093	7d Proportion Survived	Spearman-Kärber	EC50	77.52	64.86	92.64	1
05-8575-6137	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	✓ IC15	43.95	20.44	52.08	1
			✓ IC20	46.77	24.93	57.12	
			✓ IC25	49.75	29.48	62.79	
			✓ IC40	59.88	42.27	91.8	
			✓ IC50	67.74	51.5	105.4	
12-3102-9181	Mean Dry Weight-mg	Linear Interpolation (ICPIN)	IC15	62.79	33.61	84.62	1
			IC20	72.99	42.95	---	
			IC25	>160	---	---	
			IC40	>160	---	---	
			IC50	>160	---	---	

## Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
01-0574-3150	7d Proportion Survived	Control Resp	0.88	0.8	<<	Yes	Passes Criteria
01-7825-3093	7d Proportion Survived	Control Resp	0.88	0.8	<<	Yes	Passes Criteria
05-8575-6137	Mean Dry Biomass-mg	Control Resp	1.139	0.85	<<	Yes	Passes Criteria
17-8602-2611	Mean Dry Biomass-mg	Control Resp	1.139	0.85	<<	Yes	Passes Criteria
01-0574-3150	7d Proportion Survived	PMSD	0.3445	<<	0.25	No	Above Criteria
17-8602-2611	Mean Dry Biomass-mg	PMSD	0.2995	<<	0.5	No	Passes Criteria

**CETIS Summary Report**

Report Date: 27 Sep-23 00:52 (p 2 of 3)  
 Test Code/ID: P220110.103 / 06-6644-4095

**Pacific Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

**7d Proportion Survived Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.8800	0.7440	1.0160	0.8000	1.0000	0.0490	0.1095	12.45%	0.00%
20		5	0.8800	0.6579	1.1020	0.6000	1.0000	0.0800	0.1789	20.33%	0.00%
40		5	0.8000	0.4959	1.1040	0.4000	1.0000	0.1095	0.2449	30.62%	9.09%
80		5	0.4000	0.0488	0.7512	0.0000	0.8000	0.1265	0.2828	70.71%	54.55%
160		5	0.0800	-0.1421	0.3021	0.0000	0.4000	0.0800	0.1789	223.61%	90.91%
320		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

**Mean Dry Biomass-mg Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.139	0.955	1.323	0.97	1.316	0.0662	0.148	13.00%	0.00%
20		5	1.193	1.02	1.366	0.956	1.31	0.06231	0.1393	11.68%	-4.78%
40		5	1.08	0.6921	1.467	0.588	1.364	0.1396	0.3121	28.91%	5.20%
80		5	0.4252	0.03052	0.8199	0	0.896	0.1422	0.3179	74.76%	62.66%
160		5	0.0824	-0.1464	0.3112	0	0.412	0.0824	0.1843	223.61%	92.76%
320		5	0	0	0	0	0	0	0	---	100.00%

**Mean Dry Weight-mg Summary**

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	1.303	1.065	1.542	1.204	1.645	0.0859	0.1921	14.74%	0.00%
20		5	1.423	0.8884	1.957	0.956	2.08	0.1924	0.4303	30.25%	-9.18%
40		5	1.362	1.282	1.441	1.307	1.47	0.02858	0.06391	4.69%	-4.48%
80		4	1.049	0.9435	1.154	0.96	1.12	0.03306	0.06613	6.31%	19.52%
160		1	1.03			1.03	1.03	---	---	---	20.96%

**7d Proportion Survived Detail**

MD5: DB978D246AA77F3247CB927AC7063B46

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.8000	1.0000	0.8000	0.8000	1.0000
20		1.0000	0.8000	0.6000	1.0000	1.0000
40		0.4000	0.8000	1.0000	1.0000	0.8000
80		0.4000	0.4000	0.8000	0.0000	0.4000
160		0.4000	0.0000	0.0000	0.0000	0.0000
320		0.0000	0.0000	0.0000	0.0000	0.0000

**Mean Dry Biomass-mg Detail**

MD5: AD9DE13AFD855A6085B2D45550085F76

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.316	1.204	1	0.97	1.204
20		1.19	1.262	1.248	0.956	1.31
40		0.588	1.064	1.364	1.336	1.046
80		0.424	0.422	0.896	0	0.384
160		0.412	0	0	0	0
320		0	0	0	0	0

**Mean Dry Weight-mg Detail**

MD5: B41B6A557C90B6E537B67C468E72745C

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.645	1.204	1.25	1.213	1.204
20		1.19	1.577	2.08	0.956	1.31
40		1.47	1.33	1.364	1.336	1.307
80		1.06	1.055	1.12	---	0.96
160		1.03	---	---	---	---
320		---	---	---	---	---

# CETIS Summary Report

Report Date: 27 Sep-23 00:52 (p 3 of 3)  
Test Code/ID: P220110.103 / 06-6644-4095

## Pacific Topsmelt 7-d Survival and Growth Test

EcoAnalysts

### 7d Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	4/5	5/5	4/5	4/5	5/5
20		5/5	4/5	3/5	5/5	5/5
40		2/5	4/5	5/5	5/5	4/5
80		2/5	2/5	4/5	0/5	2/5
160		2/5	0/5	0/5	0/5	0/5
320		0/5	0/5	0/5	0/5	0/5

CETIS Test Data Worksheet

Report Date: 27 Sep-23 01:02 (p 1 of 2)  
 Test Code/ID: P220110.103 / 06-6644-4095

Pacific Topsmelt 7-d Survival and Growth Test

EcoAnalysts

Start Date: 14 Aug-23 15:25 Species: Atherinops affinis Sample Code: P220110.103  
 End Date: 21 Aug-23 13:50 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant  
 Sample Date: 10 Jan-22 Material: Copper Sample Station: P220110.103

Conc-ug/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Weightmg Total	Weightmg Tare	Pan Count	Notes
0	D	1	26	5							4	59.02	52.44	4	
0	D	2	12	5							5	57.23	51.21	5	
0	D	3	21	5							4	56.85	51.85	4	
0	D	4	23	5							4	54.88	50.03	4	
0	D	5	30	5							5	69.97	63.95	5	
20		1	3	5							5	59.4	53.45	5	
20		2	15	5							4	57.71	51.4	4	
20		3	4	5							3	61.41	55.17	3	
20		4	1	5							5	64.52	59.74	5	
20		5	14	5							5	69	62.45	5	
40		1	18	5							2	59.24	56.3	2	
40		2	27	5							4	54.9	49.58	4	
40		3	5	5							5	61.88	55.06	5	
40		4	22	5							5	57.59	50.91	5	
40		5	25	5							4	59.54	54.31	4	
80		1	7	5							2	56.9	54.78	2	
80		2	29	5							2	53.31	51.2	2	
80		3	6	5							4	53.61	49.13	4	
80		4	19	5							0	0	0		
80		5	17	5							2	58.42	56.5	2	
160		1	11	5							2	169.91	167.85	2	
160		2	16	5							0	0	0		
160		3	13	5							0	0	0		
160		4	10	5							0	0	0		
160		5	24	5							0	0	0		
320		1	28	5							0	0	0		
320		2	9	5							0	0	0		
320		3	20	5							0	0	0		

CETIS Test Data Worksheet

Report Date: 27 Sep-23 01:02 (p 2 of 2)  
 Test Code/ID: P220110.103 / 06-6644-4095

Conc-µg/L	Code	Rep	Pos	# Exposed	1d Survival	2d Survival	3d Survival	4d Survival	5d Survival	6d Survival	7d Survival	Total Weight-mg	Tare Weight-mg	Pan Count	Notes
320		4	2	5							0	0	0		
320		5	8	5							0	0	0		

### 7 Day Chronic Survival and Growth Test

Toxicant:	Copper Chloride
Ref Tox ID:	922010, 103
Lot #:	MKCK7155
Protocol:	TOX002
Replicates:	5

Date Test Started:	8/14/2023
Date Test Ended:	8/21/2023
Matrix:	Liquid
Species:	<i>Atherinops affinis</i>
No. of Org. per Chamber:	5

	Conc. (µg/L)	Meter #:	DO (mg/L) (>4.0)	Meter #:	Temp (°C) (20±1°C)	Meter #:	Salinity (ppt) (30±2ppt)	Meter #:	pH (6 - 9)
<b>Day 0 (Stock)</b>	Control	9	7.6	9	18.9	9	30	9	7.9
Date: 8/14/23	20		7.7		18.8		30		7.9
Time: 1015	40		7.7		18.9		30		7.9
Technician: J1	80		7.7		18.9		30		7.9
Feed: TW	160		7.7		19.0		30		7.9
(500 nauplii/chamber)	320		7.7		19.0		30		7.9
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6			
Temperature (OLD)	20.0	19.8	20.2	20.1	19.9	20.2	20.2		
Temperature (NEW)	19.8	19.4	19.5	19.7	19.3	19.4			
<b>Day 7</b>	Control	9	6.6	9	20.4	9	31	9	7.8
Date: 8/21/23	20		6.2		20.6		31		7.8
Time: 1330	40		6.5		20.5		31		7.9
Replicate No.: 1	80		6.8		20.5		31		8.0
Technician: TW	160		6.9		20.5		31		8.0
	320		—		—		—		—

**Dilution Preparation (Serial dilute by 50%)**

CuCl <sub>2</sub> *2H <sub>2</sub> O Stock Solution:	Target Stock Solution Conc.	Volume of Stock Solution	Amt. of Toxicant
400,000 µg/L	320 µg/L	2500 g	2 g
400,000 µg/L	160 µg/L	2500 g	1 g

Day	Date	Init.	Highest Conc.	Day	Date	Init.	Highest Conc.
0	8/14/23	J1	320	4	8/18/23	0 + 60 J1	160
1	8/15/23	J1	320	5	8/19/23	NL	160 µg/L
2	8/16/23	TW	320	6	8/20/23	NL	160 µg/L
3	8/17/23	NL	160 µg/L				

Start Time:	1525 LG/TW
End Time:	1350 TW
Test Location:	Bath 1
Dilution Water Batch:	FSW081323.01

Supplier:	ABS
Organism Batch:	ABS081123.01 Age: 14 days
Chamber Size/Type:	20 oz. cup
Exposure Volume:	250 mL

016 J1 8/15/23 mtrms, J1 8/18/23

**7 Day Chronic Survival and Growth Test**

Toxicant: Copper Chloride  
 Ref Tox ID: P22010-103

Date Test Started: 8/14/23  
 Date Test Ended: 8/21/23

Species: *Atherinops affinis*

Concentration (µg/L)	Rep	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
		Date: 8/15/23		Date: 8/16/23		Date: 8/17/23		Date: 8/18/23		Date: 8/19/23		Date: 8/20/23		Date: 8/21/23	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	5	0	5	0	5	0	4	1	4	0	4	0	4	0
	2	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	3	5	0	5	0	5	0	5	0	5	0	4	1	4	0
	4	5	0	5	0	5	0	5	0	2 <sup>5</sup> 4 (20)	1	4	0	4	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
20	1	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	2	5	0	5	0	5	0	5	0	5	0	2 <sup>5</sup> 4	1	4	0
	3	3	2	3	0	3	0	3	0	3	0	3	0	3	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0
40	1	5	0	5	0	5	0	3	2	3	0	3	0	2	1
	2	5	0	5	0	5	0	5	0	4	1	4	0	4	0
	3	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	4	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	5	5	0	5	0	5	0	4	1	4	0	4	0	4	0
80	1	5	0	5	0	4	1	4	0	2	2	2	0	2	0
	2	5	0	5	0	4	1	2	2	2	0	2	0	2	0
	3	5	0	5	0	4	1	4	0	4	0	4	0	4	0
	4	5	0	5	0	3	2	3	0	2	1	1	1	0	1
	5	5	0	5	0	5	0	4 <sup>20</sup>	1	4	0	3	1	2	1
160	1	4	1	2 <sup>4</sup> 3 1 <sup>0</sup>		2	1	2	0	2	0	2	0	2	0
	2	5	0	4	1	2	2	1	1	0	1				
	3	4	1	3	1	2	1	0	2						
	4	4	1	3	1	3	0	1	2	0	1				
	5	4	1	3	1	2	1	1	1	0	1				
320	1	0	5												
	2	1	4	0	1										
	3	0	5												
	4	0	5												
	5	0	5												
Feeding: 250 am/500 pm (artemia/chamber)	AM	TW		TW		DND		JL		NL		NL		None	
	PM	TW		TW		NL/JL		JL		NL		NL		None	

① Fed 1/2 to chambers with 50% mort. TW, 8/15/2023, TW, 8/16/23, PM-8/17/23  
 ② I.E, TW 8/16/23, NL 8/19/23, NL 8/20/23

NL 8/19/23, NL 8/20, 1 of 1  
 NL 8/20

**7 Day Chronic Survival and Growth Test**

Toxicant: Copper Chloride  
 Ref Tox ID: P220110.103

Date Test Started: 8/14/23  
 Date Test Ended: 8/21/23  
 Species: *Atherinops affinis*

Concentration (µg/L)	Replicate	Boat Number	Weight Empty Boat (mg)	Weight Boat & Animals (mg)	Pan Count
Control	1	1	52.44	59.02	4
	2	2	51.21	57.23	5
	3	3	51.85	56.85	4
	4	4	50.03	54.88	4
	5	5	63.95	69.97	5
20	1	6	53.45	59.40	5
	2	7	51.40	59.71	4
	3	8	55.17	61.41	3
	4	9	59.74	64.52	5
	5	10	62.45	69.00	5
40	1	11	56.30	59.24	2
	2	12	49.58	54.90	4
	3	13	55.06	61.88	5
	4	14	50.91	57.59	5
	5	15	54.31	59.54	4
80	1	16	54.78	56.90	2
	2	17	51.20	53.31	2
	3	18	49.13	53.61	4
	4	19	56.90	—	—
	5	20	56.50	58.42	2
160	1	21	167.85	169.91	2
	2				
	3				
	4				
	5				
320	1				
	2				
	3				
	4				
	5				

Date/Time in oven: [Init.]	8/20 1627	NL	8/21 1430	TW
OvenTemp: [Init.]	160	NL	100	TW
Date/Time removed from oven (and placed in desiccator): [Init.]	8/21/23 0848	UG	8/22/23 1055	TW
Weight date and time (removed from desiccator): [Init.]	8/21/23 9000	UG	8/22/23 1506	LG

OIE-NL 8/20/23 X1: 56.85 mg

1	29
2	24
3	14
4	28
5	19
6	18
7	1
8	15
9	13
10	27
11	12
12	8
13	9
14	20
15	4
16	11
17	16
18	21
19	3
20	17
21	6
22	22
23	25
24	26
25	5
26	23
27	30
28	10
29	7
30	2

# P220110.103

Topsmelt  $\text{CuCl}_2$  RT Chronic  
8/14/23 - 8/21/23

## **APPENDIX B**

Chain of Custody  
Sample Receipt Form

# CHAIN OF CUSTODY



EcoAnalysts, Inc.  
4770 NE View Dr., Port Gamble, WA. 98364  
Tel: (360) 297-6040

Destination: <b>EcoAnalyst</b>		Sample Originator (Organization): <b>Alon Asphalt Company</b>		Report Results To: <b>Alon Asphalt Company</b>		Phone: <b>425-381-3770</b>																	
Destination Contact: <b>Marisa Seibert</b>		PERSON WHO COLLECTED SAMPLE: <b>Mark Thomas</b>		Contact Name: <b>Mark Thomas</b>		Fax:																	
Date: <b>8/21/23</b>		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Email: <b>mark.thomas@delekus.com</b>																	
Turn-Around-Time:		Phone: <b>425-381-3770</b>		Analyses:		Involving To:																	
Project Name: <b>Alon Asphalt Company</b>		Fax:		<table border="1" style="width:100%; text-align: center;"> <tr> <td style="width: 50px;">ACUTE</td> <td style="width: 50px;">CHRONIC</td> <td style="width: 50px;"></td> </tr> <tr> <td style="text-align: center;">X</td> <td style="text-align: center;">X</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> </tr> </table>		ACUTE	CHRONIC							X	X							Comments or Special Instructions: <b>36°F</b>	
ACUTE	CHRONIC																						
X	X																						
Contract/PO:		E-mail: <b>mark.thomas@delekus.com</b>				Preservation		Sample Temp Upon Receipt		LAB ID													

No.	Sample ID	Secondary ID: Replicate, X of Y, etc.	Matrix	Volume/Mass	Date	Time	ACUTE	CHRONIC							
1	OUTFALL #1 - GRAB		H2O	20L	8/21/23	00:00	X	X					Ice	2.1	P230821.01
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															

Relinquished by:		Received by:		Relinquished by:		Received by:		<b>Matrix Codes -</b> FW = Fresh Water SB = Salt & Brackish Water SS = Soil & Sediment TS = Tissue
Print Name: <b>Mark Thomas</b>		Print Name: <b>Thomas Wilson</b>		Print Name:		Print Name:		
Signature: <i>[Signature]</i>		Signature: <i>[Signature]</i>		Signature:		Signature:		
Affiliation: <b>Alon Asphalt Company</b>		Affiliation: <b>Eco Analyst Inc.</b>		Affiliation:		Affiliation:		
Date/Time: <b>8/21/23 0900</b>		Date/Time: <b>8/21/2023 1305</b>		Date/Time:		Date/Time:		

# CHAIN OF CUSTODY



EcoAnalysts, Inc.  
4770 NE View Dr., Port Gamble, WA. 98364  
Tel: (360) 297-6040

Destination: <b>EcoAnalyst</b>		Sample Originator (Organization): <b>Alon Asphalt Company</b>		Report Results To: <b>Alon Asphalt Company</b>		Phone: <b>425-381-3770</b>								
Destination Contact: <b>Marisa Seibert</b>		PERSON WHO COLLECTED SAMPLE: <b>Mark Thomas</b>		Contact Name: <b>Mark Thomas</b>		Fax:								
Date:		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Email: <b>mark.thomas@delekus.com</b>								
Turn-Around-Time:		Phone: <b>425-381-3770</b>		Analyses:		Invoicing To:								
Project Name: <b>Alon Asphalt Company</b>		Fax:		ACUTE CHRONIC			Comments or Special Instructions: <b>33<sup>rd</sup> E 0900</b>							
Contract/PO:		E-mail: <b>mark.thomas@delekus.com</b>						Preservation	Sample Temp Upon Receipt	LAB ID				
No.	Sample ID	Secondary ID: Replicate, X of Y, etc.	Matrix	Volume/Mass	Date	Time								
1	OUTFALL #1 - GRAB		H2O	20L	8/23/23	1230 PM	X	X				Ice	1.0°C	P230823-01
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														

Relinquished by:		Received by:		Relinquished by:		Received by:		Matrix Codes FW = Fresh Water SB = Salt & Brackish Water SS = Soil & Sediment TS = Tissue
Print Name: <b>Mark Thomas</b>		Print Name: <b>Nicole Lundgren</b>		Print Name:		Print Name:		
Signature: <i>[Signature]</i>		Signature: <i>[Signature]</i>		Signature:		Signature:		
Affiliation: <b>Alon Asphalt Company</b>		Affiliation: <b>ECO A</b>		Affiliation:		Affiliation:		
Date/Time: <b>8/23/23 1000</b>		Date/Time: <b>8/23 1117</b>		Date/Time:		Date/Time:		

# CHAIN OF CUSTODY



EcoAnalysts, Inc.  
4770 NE View Dr., Port Gamble, WA. 98364  
Tel: (360) 297-6040

Destination: <b>EcoAnalyst</b>		Sample Originator (Organization): <b>Alon Asphalt Company</b>		Report Results To: <b>Alon Asphalt Company</b>		Phone: <b>425-381-3770</b>					
Destination Contact: <b>Marisa Seibert</b>		PERSON WHO COLLECTED SAMPLE: <b>Mark Thomas</b>		Contact Name: <b>Mark Thomas</b>		Fax:					
Date: <b>8/25/23</b>		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Address: <b>20555 Richmond Beach Dr NW Shoreline, WA 98177</b>		Email: <b>mark.thomas@delekus.com</b>					
Turn-Around-Time:		Phone: <b>425-381-3770</b>		Analyses:		Involving To:					
Project Name: <b>Alon Asphalt Company</b>		Fax:		ACUTE CHRONIC							
Contract/PO:		E-mail: <b>mark.thomas@delekus.com</b>						Comments or Special Instructions:			
No.	Sample ID	Secondary ID: Replicats, X of Y, etc.	Matrix	Volume/Mass	Date	Time	ACUTE	CHRONIC	Preservation	Sample Temp Upon Receipt	LAB ID
1	OUTFALL #1 - GRAB		H2O	20L	8/25/23	1230 <del>8:00</del>	X	X	ICE	0.1°C	P230825.01
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
Relinquished by:			Received by:			Relinquished by:			Received by:		
Print Name: <b>Mark Thomas</b>			Print Name: <b>Nicole Lundgren</b>			Print Name:			Print Name:		
Signature:			Signature:			Signature:			Signature:		
Affiliation: <b>Alon Asphalt Company</b>			Affiliation: <b>ECO A.</b>			Affiliation:			Affiliation:		
Date/Time: <b>8/25/23 1000 Am</b>			Date/Time: <b>8/25/23 1250</b>			Date/Time:			Date/Time:		
<div style="float: right;"> <p>Matrix Codes</p> <p>FW = Fresh Water</p> <p>SB = Salt &amp; Brackish Water</p> <p>SS = Soil &amp; Sediment</p> <p>TS = Tissue</p> </div>											

## SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:	
Alon Asphalt	Outfall #1-Grab	P230821.01	P230823.01	P230825.01
Project:				
Alon Asphalt Company				
Date/Time Received:	8/21/23, 1305	8/23/23, 1117	8/25/23, 1250	
Airbill #:	Courier	Courier	Courier	
Shipper Tracking Information Kept for Records: (Y/N/NA)	NA	NA	NA	
Collection Date/Time:	8/21/23, 00:00	8/23/23, 00:30	8/25/23, 00:30	
Sample Holding Time (must be ≤36 hours at test initiation)	✓	✓	✓	
Condition of Shipping Container:	Good	Good	Good	
Type and Capacity of Sample Container:	20L Cubi	20L cubi	20L cubi	
Total Sample Volume (L):	20L	cubi not filled @ completely management notified	~18L	
Condition of Sampling Container:	Good	Good	Good	
Sample Container Appropriate: (Y/N)	Y	Y	Y	
Custody Seals Intact: (Intact/Broken/Not Present)	Not Present	Not present	NP	
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)	Y	Y	Y	
Sampler's Name Present on COC Form: (Print Name/Not Present)	Mark Thomas	Mark Thomas	Mark Thomas	
Color:	Clear	Clear	pale yellow	

### TAKE THE FOLLOWING MEASUREMENTS UPON ARRIVAL

LAB ID	Meter #	Temp. (°C) * (0-6°C)	Meter #	Dissolved Oxygen (mg/L)	Meter #	pH	Meter #	Cond. (µS/cm)	Meter #	Sal. (ppt)	Hardness (mg CaCO <sub>3</sub> /L)	Alkalinity (mg CaCO <sub>3</sub> /L)	Total Chlorine (mg/L)	Total NH <sub>3</sub> (mg/L)	Tech
P230821.01	T21	2.1	8	10.1	8	7.3	8	215	8	0.072	—	—	0.02	① 0.004	TW/MS
P230823.01	E1	1.0	7	10.3	7	6.7	7	216.9	7	0.116	—	—	0.03	① 0.00	TI/NL
P230825.01	N6	0.1	9	9.6	9	7.5	9	256	9	0.169	—	—	0.01	① 0.00	NL/MS

\*Notify project manager or study director of temperatures above 6°C or ≥36 hours holding time. Client must be notified ASAP.

If there are sample receipt problems, complete the following:	
Reason for unacceptability:	
Name of Client Contact:	Contacted by:
Client Response and/or Action to be Taken:	Date Action Taken:

① NH<sub>3</sub> sample preserved for later analysis - MS 8/21, TI 8/23/23, NL 8/25/23  
 ② Cubi not filled all the way up. management notified - TI 8/23/23